

POST WAR COLLEGE & UNIVERSITY – VOL. 5 (A)
Research theses and Grants

THE POST-WAR YEARS

THE G. I. BILL
Seton Hall/ U. of Delaware/ U. of Pennsylvania

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Seton Hall/ U. of Delaware/ U. of Pennsylvania

I enrolled in the September 1946 class at Seton Hall College and completed my Bachelor of Science degree in Chemistry in September of 1948.

I entered the Univ. of Delaware in September of 1948 and completed my Master of Science in Biology degree in September of 1950.

I then entered the Univ. of Pennsylvania and completed my Doctor of Philosophy degree in Biology in May of 1955.

My professional career began in September of 1955 as an assistant professor of Biology at Wilkes College in Wilkes-Barre, Pennsylvania. I became a full Professor of Biology in 1960, Academic Dean in 1963, and President in 1970.

I served as President of Wilkes until June of 1975 when I assumed the position of President of the Commission for Independent Colleges and Universities of Pennsylvania. I served in that role until retirement in December of 1990.

F/M
N.B.

I was in this group of students at Seton Hall in Fall 1946

Veterans of Battles

The G.I. Bill fueled the post-war enrollment boom at Seton Hall.

A boisterous group of young men, barely out of high school but already veterans of a world war, liked to gather at Barry's Tavern in Orange, New Jersey, to hoist their beers and trade tales of military prowess. Bartender George Barry, a man with little formal education, would admonish them to get on with life and take advantage of G.I. education benefits.

One night, there was a phone call at the tavern for Jim Morrissey, one of the young revelers.

"It was a Father Carey from Seton Hall, whom I had never heard of, ordering me to meet him on the campus the following day. Of course I did what he said, and before I knew it I was enrolled at the college," says Morrissey '50, who was a replacement in the 5th Marine Division on Iwo Jima. He later found out that the bartender was friendly with Father Carey [Reverend James Carey] and, concerned for the young vet's future, had tipped off the priest about him.

Father Carey's pursuit of Morrissey is just one example of Seton Hall's aggressive campaign nearly 50 years ago to bring World War II veterans to South Orange as students. Seton Hall's interest in veterans continued, many who served in war and in peacetime would come to campus to earn their degrees with the aid of government assistance.

In September 1945, Seton Hall's president, Monsignor James F. Kelley, announced that the institution would open its doors to all qualified veterans. He vowed that the tiny college would do everything possible to help the returning G.I.s earn their degrees.

The proclamation gained national attention for Seton Hall through newspaper articles in the *New York Times*, the *Newark Star-Ledger* and the *Newark Evening News*.

By Patricia Monahan Sutter '82



Monsignor James F. Kelley, Seton Hall's president, honored the University's war heroes during the dedication of the "vet village" in 1946. He was among the first college presidents to encourage servicemen to use the G.I. Bill to earn their degrees.

At Seton Hall, enrollment swelled from several hundred students to several thousand and helped the college earn university status in 1950, when it then graduated its largest class to date.

As soon as veterans stepped out of the service, Seton Hall was there to greet them. In *Memoirs of Monsignor (Doc) J. F. Kelley*, his 1987 autobiography, the president wrote that when the war ended, Seton Hall set up registration centers for the Army at Fort Dix, New Jersey, and for the Navy at the Brooklyn Naval Yard.

Thousands of copies of "The Veteran Goes to College," a brochure detailing Seton Hall's admission requirements, veterans' rehabilitation services and educational opportunities, were published. For those who did not finish high school, the institution offered a

personally supervised, intensive course to enable would-be applicants to obtain high school equivalency certificates.

Dormitories quickly filled. Beds were put in the school's squash courts, on the gymnasium floor and even in the empty swimming pool.

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and Book



The booming Seton Hall of the early post-war years wasn't the college it used to be. Gone were the days of freshmen who wore beanies, and in marched the seasoned veterans-turned-students who ranged in age from 19 to 29. Many were married. Young wives and mothers with children became familiar sights on the all-male campus.

One year after World War II ended, Seton Hall's enrollment soared from 140 students to 902, and 95 percent of them were veterans. In 1947, 2,500 students enrolled, and by 1949, 3,000 students were on campus. In all, more than 2,000 veterans attended Seton Hall. The faculty increased by 50 percent as professors were recruited from Harvard, Yale, Columbia and Fordham.

The population explosion spurred a \$325,000 program to erect three brick buildings. In the short-term, however, 10 wooden structures, obtained from Fort Monmouth, New Jersey, were used as temporary classrooms; each was about 20-feet-by-100-feet. And the "vet village" rose. About 200 resident stu-

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The March to the Nation's Classrooms

Veterans marched home from war
and into Seton Hall and other col-
leges across the nation as a result
of the Serviceman's Readjustment Act,
better known as the "G.I. Bill of Rights."
Signed by President Franklin D.
Roosevelt on June 22, 1944, the bill off-
set some of the dire predictions of
unemployment and depression for the
post-war years.

Among the bill's numerous benefits
were paid education and training; loan
guaranty for homes, farms and busi-
nesses; unemployment pay and assis-
tance in finding jobs; provision of
building materials for Veterans Admin-
istration (VA) hospitals; and military
review of dishonorable discharges.

To be eligible for G.I. education ben-
efits, veterans had to have served at least
90 days after Sept. 16, 1940, and could
not have been dishonorably discharged.
They were entitled to one year of full-
time training, plus a period equal to their
time in the service, up to 48 months.

At a time when Seton Hall's tuition
was \$10 per credit, the VA paid educa-
tional institutions up to \$500 a year for
tuition, books, fees and other training
costs, and also provided unmarried vet-
erans with allowances of up to \$50 a
month. Those with dependents received
higher allotments.

By the time the \$14.5 billion program
ended in 1956, more than 2.2 million of
the 15.5 million World War II veterans
had attended college, according to VA
statistics. Participation in the program
peaked in 1947, when veterans accounted
for 49 percent of U.S. college enrollment.

—Patricia Monahan Sutter '82



Veterans' groups are commemorating the 50th anniversary of the
end of World War II with an emblem depicting the Honorable
Service Lapel Pin. Affectionately known as the "Ruptured Duck,"
the brass pin was issued to more than 12 million vets.



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Actor Pat O'Brien helped Seton Hall dedicate the new veterans' village in the fall of 1946. The first building was named after Lt. John Ruthenberg '44, the first soldier from Seton Hall to be killed in the war; he had been a member of Seton Hall's famous "Wonder Five" basketball team in the early '40s.

Retired and living in Rumson, New Jersey, the 92-year-old Monsignor Kelley has fond remembrances of post-World War II campus life.

"As long as the veterans were good students, they were entitled to be there, and we were thrilled to have them. They brought in a spirit that hadn't been there before," he says. "Other colleges didn't take advantage of the opportunities to have them. It was wonderful to see them come in and start their studies."

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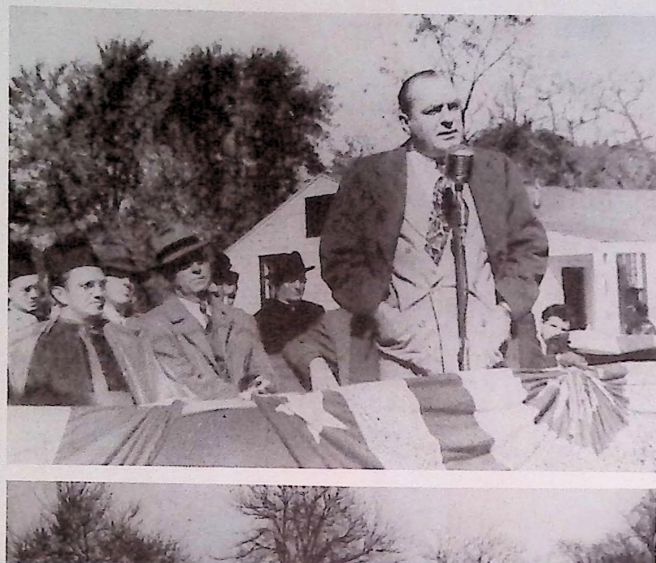
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Bob Pocelinko
and Pete Eckert

Bob and Ed
jazz up to state park driving through
the woods to eat lunch!



"Seton Hall
1943
CANTONMENT"

STAPLES



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When I was an undergraduate in 1943 and following WWII in 1946 to 1949 the library located at the far end of this main building was my favorite study spot.

Great memories of classmates Bob Pocelinko, Ed Wolski, Bill Callahan and Pete Eckert

Bob and Ed shared rides in the jeep up to state park driving through the woods to eat lunch!



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Morrissey attended classes in prefabricated, metal-framed, semicircular Quonset huts. "We were still catching our breath from the war, and sometimes it was hard to buckle down and do schoolwork," recalls Morrissey, who arranged his schedule to work part-time.

Jim Cooke '50, who had served in Germany as an infantryman, went to classes in boiler rooms. "We didn't have

Patricia Monahan Sutter '82 is a frequent contributor to the Seton Hall University Magazine.

University of Delaware
President Form 4



STAPLES

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(Top) Actor Pat O'Brien spoke at the dedication ceremonies for Seton Hall's "vet village." (Above) From barracks to dormitories: For the servicemen-turned-students, perhaps the new living quarters were stark reminders of military life.

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Morrissey notes that priests, some of whom were World War II chaplains, provided the guidance and discipline needed for the young men to complete their college education. Thanks to Seton Hall's supportive environment, many young men who had never dreamed of going to college were able to take advantage of the government's benefits. Says Cooke, "I didn't grow up expecting to go to college, and wouldn't have gone without the bill."

Likewise for Morrissey: "Before the war I wasn't planning to go to college. I couldn't afford it and I didn't think my grades were good enough. Without the opportunity offered by the government and Seton Hall, I would never have gone."

Patricia Monahan Sutter '82 is a frequent contributor to the Seton Hall University Magazine.



STAPLES

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University of Delaware
President Form 4

Name: Francis

Department: Biol.

School: Arts & Sciences

Your status on the
of Trustees as follows:

Title: Research

Compensation: \$200 per mo

Period of Appointment

Will you kindly inc
this appointment retain

Accepted Francis
Declined

STAPLES

NOTICE OF REAPPOINTMENT

Name: Francis Joseph Micheline

Department: Biological Sciences

School Arts and Science

Your status on the staff of the University has been approved pending final confirmation by the Board of Trustees as follows:

Title Research Fellow

Compensation \$1400.00 for part time

(\$200 per mo. July 1 - Aug. 31; \$100 per mo. ^(full, part) Sept. 1 - June 30)

Period of Appointment: From July 1, 1949 to June 30, 1950

Will you kindly indicate by signature on the two copies of this letter your acceptance or declination of this appointment retaining the original for your files and returning the carbon copy to me?

William S. Carson
President

Accepted Francis J. Micheline

Declined _____

University of Delaware
President Form 4

Summer Research

NOTICE OF REAPPOINTMENT

Name: Francis J. Michelinì

Department: Biological Sciences

School Arts and Sciences

Your status on the staff of the University has been approved pending final confirmation by the Board of Trustees as follows:

Title Graduate Assistant

Compensation \$400.00 for full time
(full, part)

Period of Appointment: From July 1, 1951 to August 31, 1951

Will you kindly indicate by signature on the two copies of this letter your acceptance or declination of this appointment retaining the original for your files and returning the carbon copy to me?

January 21, 1952

Name: Michelinì, Francis
Title: Research Fellow

During the year 1951 you received a grant from the University of Delaware of \$ 400. as a fellowship. The source of these funds was the Damon Runyon Memorial Fund.

Inasmuch as this fellowship was solely for the purpose of enabling you to conduct graduate study and no services were required of you by the University or the organization supplying the funds and no special benefits were to go to any private person or private organization (such as patent rights), the University considered your fellowship to be a gift and not taxable according to G.C.M. 5881, VIII-1 CB 68.

A. P. Colburn
A. P. Colburn
Provost

University of Delaware
Newark, Delaware

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ending final confirmation by the Board

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August 31, 1951

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January 21, 1952

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P. Colburn
P. Colburn
Provost

A Comparative Study of the Effects
of X-radiation and Nitrogen Mustard
on Dividing and Non-dividing Cells in
Zea Mays Seedlings

by

Francis J. Michelini

A thesis submitted to the Faculty of the University
of Delaware in partial fulfillment of the requirements for the
degree of Master of Science.

June 1950

STAPLES

University of Delaware
Master's Degree Thesis – June, 1950

Master's Degree Awarded September 21, 1950

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SUMMARY

Experimental work was done to determine the relation of mitosis in the corn seedling to sensitivity to X-rays and methyl bis (beta-chloroethyl) amine hydrochloride, referred to as HN_2 .

Two methods were employed to provide tissue under conditions of normal cell division and an absence or decrease of cell division, while at the same time keeping other variables at a minimum. Method A involved the use of 11 and 12 hr. embryos where no cell division was present and 15 and 16 hr. embryos which contained actively dividing cells. Method B involved the use of a mitotic inhibitor, 3,5 dinitro-o-cresol, (DNC) which decreased cell division in seedlings approximately 70%.

In both methods the following criteria were used as tests for sensitivity:

1. The percentage of roots dying.
2. The percentage of roots showing laterals at the end of the experiment.
3. The time of appearance of laterals in cases where all groups had laterals at the end of the experiment.

By both methods the data show:

- A) The sensitivity to HN_2 was not influenced by the presence or absence of cell division.
- B) The sensitivity to X-rays was greatly increased when mitotic activity was present.

While there are many similarities in the effects of HN_2 and X-rays, there is a marked difference between these agents when their effect on this organism in different mitotic conditions is tested.

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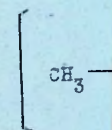
INTRODUCTION

Much attention has been given to mustard compounds since 1941 when Charlotte Auerbach discovered that these chemicals produced mutations in Drosophila similar to those produced by x-rays. Further studies have shown more points of similarity between the action of these compounds and the effects of radiation. Bryson (4) reported that one particular strain of bacteria (Escherichia coli) which was resistant to radiation was also relatively resistant to the action of nitrogen mustard. Gilman and Philips (10) stressed the fact that both nitrogen mustard and x-rays have an effect upon the divisional mechanism of the cell, resting cells being comparatively unaffected by treatment with both agents while dividing cells are highly sensitive. Gillette and Bodenstein (9) working with Amblystoma embryos reported that regions of actively proliferating tissue were more sensitive to nitrogen mustard than regions with less cell division. Henshaw and Francis (12) found that when wheat seedlings were exposed to x-rays just before or just after the onset of mitosis there was a sudden increase in sensitivity in the latter group.

The literature shows a dearth of evidence where the same organism in varying mitotic stages is exposed to both agents and the results compared. Comparison of the sensitivity of dividing and resting cells to nitrogen mustard and x-rays was therefore selected for the present investigation. In the study of such a problem germinating Zea Mays was an ideal material since it could be exposed to either agent with relative ease. Large numbers could be used and environmental conditions readily controlled. Again, it was possible to determine quite accurately the prevalence of cell division in the experimental material. This was quite an important factor since the results are interpreted in the light of the presence and absence of cell division.

Agents Used

The mustard compound used was nitrogen mustard, hydrochloride, common name HN₂ (4). It has the following chemical structure. Its mode of action is usually cytotoxic. Structurally it is represented by

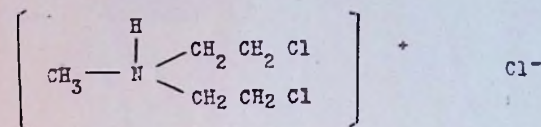


Since this compound is one of the most potent and safest of the various agents used in the experiments, a solution of the crystalline form was prepared by then taking 500 ml. of water and adding a concentration used in the experiments. These solutions were prepared at the final temperature of the experiment and were immersed in the water bath. They were placed in the water bath of solution used during the experiment. active over the 30 minutes. A 0.001% concentration was used in solution at pH 7.0 and was thoroughly in solution.

mustard compounds since 1941 when these chemicals produced mutations in *Y* x-rays. Further studies have shown the action of these compounds and the fact that one particular strain of *Y* resistant to radiation was also sensitive to nitrogen mustard. Gilman and Philips found that nitrogen mustard and x-rays have an effect on *Y* cells, resting cells being comparatively resistant while dividing cells are highly sensitive. Working with *Amblystoma* embryos, dividing tissue was more sensitive than non-dividing tissue. Less cell division. Henshaw and Phillips were exposed to x-rays just before division and there was a sudden increase in the number of cells. Evidence where the same organism in both agents and the results compared. In dividing and resting cells to nitrogen mustard. Used for the present investigation. Using *Zea Mays* was an ideal material to work with relative ease. Large numbers of plants readily controlled. It was found that the prevalence of mutation was quite an important factor. This was quite an important factor in the light of the presence

Agents Used

The mustard compound used was methyl bis (beta-chloroethyl) amine hydrochloride, commonly referred to as MBA (9) and more recently as HN₂ (4). It has the empirical formula C₅ H₁₂ Cl₂ and its mechanism of action is usually attributed to an active onium cation (Osgood, 17). Structurally it is represented as:



Since this compound is water soluble it was found to be the most convenient and safest of the various mustard compounds for this work. The solutions used in the experiments were always made up in the same manner, using 20 mg. of the crystalline HN₂ salt in 1000 ml. to make a 1.04 x 10⁻⁴M solution, then taking 500 ml. of this solution in 1000 ml. to make the 5.02 x 10⁻⁵M concentration used in some of the experiments. Where the 5.2 x 10⁻⁶M solution was needed, 50 ml. of the 1.04 x 10⁻⁴M was made up to 1000 ml. These solutions were all made up with distilled water at 30° C. and the final temperature of the solutions ranged from 27 to 30° C. The seedlings were immersed in the solution for the time required and during exposure were placed in the incubator to keep the temperature at 30° C. The volume of solution used during exposure was kept constant and was shown to be active over the 30 minute period used (Russell, 19). She had found the 0.001% concentration to be active over a minimal 6 hour period when made up in solution at pH 5.6. After treatment the seedlings were washed thoroughly in running tap water for five minutes.

MATERIALS AND METHODS

5.

Zea Mays Linnaeus, of the U.S. #13 strain, was used in these studies. At the start of each experiment the seed was routinely treated for five minutes with a 0.05% mercuraphen solution to kill mold spores, soaked in water for one hour, spread between layers of moist paper towelling in germinating dishes and placed in an incubator at 30° C. The germinated corn was ready for treatment at varying times depending on the method employed in each experiment. After treatment they were placed in jars of moist sphagnum which had been previously prepared. These were tall 600 ml. beakers lined with filter paper and loosely packed with moist sphagnum. This moss was previously treated with Sorensen's phosphate buffer (pH 6.4) to bring up to and maintain a pH of approximately 5.0. These beakers were kept covered with petri dishes and autoclaved prior to use. Fifteen to eighteen seedlings were placed in each beaker with the root between glass and paper; they were then grown at 30° C. for about 6 days. This technique permitted the measuring of the primary root length of each individual twice daily, and the observation of the time of appearance of laterals, without disturbing the seedlings. At the same time it made available a record of individual root growth.

For all cytological work standard methods were employed as described by Johansen (13). Navashin's fixative and the tertiary butyl alcohol (TBA) method of dehydration were used. Paraffin blocks were cut longitudinally into sections 10 microns in thickness and subsequently stained with Heidenhain's hematoxylin. Three to four median sections of each root were checked for dividing cells.

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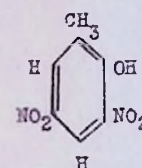
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seedlings were placed in each beaker with
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The X-ray machine used in these experiments was operated at 120 kv.,
10 ma, with a 1 1/2 mm. aluminum filter and delivered approximately 75 r
per minute. The corn seeds were placed embryo side up in a petri dish
with a cellophane cover to prevent excessive dehydration. When two
different groups were being exposed, one set was marked for identification
with india ink and both were then treated at the same time.

One phase of these studies involved the use of 3,5 dinitro-o-cresol
(DNC) as a reversible mitotic inhibitor. This compound is water soluble,
has the empirical formula $C_7 H_6 N_2 O_5$ and may be represented structurally:



An approximately $10^{-5}M$ solution was used; this was made up by dissolving
20 mg. of the crystalline DNC in 1 liter of solution to make a $10^{-4}M$
solution and then taking 50 ml. of this solution in 500 ml. to make the
 $10^{-5}M$ concentration.

Methods

Two different methods were employed for obtaining conditions in the
experimental material where there was cell division present in one case
and absent in the other. For clarity these will be referred to as
"Method A" and "Method B" throughout the remainder of this paper.

6.
"Method A" - Use of embryos germinated for different periods of time.

As a preliminary to this problem observations of stained sections made from the radicles of corn embryos showed that after 11 hours germination at 30° C. no mitotic figures could be found, but after 15 hours of germination many cells were dividing. About thirty 11 hr. embryos were sectioned and none showed cell division while of thirty 15 hr. embryos sectioned all showed the presence of dividing cells. During the course of each experiment an average of nine embryos of each set were prepared and examined for the presence or absence of division. When the supply of 1948 seed was exhausted it was found that the 1949 seed (of the same strain) seemed to germinate more slowly. After checking the slides made from this crop it was found necessary to use a 16 hr. germinating period to insure the presence of cell division. A 12 hr. germinating period still resulted in the absence of cell division. In subsequent experiments 12 and 16 hour embryos were used for the two conditions. A total of sixty 11 hr. and 12 hr. embryos were checked and failed to show the presence of division while eighty-five 15 and 16 hr. embryos checked showed actively dividing cells.

"Method B" - Treatment with 3,5 dinitro-o-cresol (DNC) to inhibit cell division.

As a check on results obtained in the method outlined in A, the following procedure was employed to obtain a group of seedlings where cell division was decreased in order to contrast their behavior with a group where cell division was normal. Clowes and Krah1 (6) working with sea urchin eggs found DNC to be a reversible mitotic inhibitor.

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ated for different periods of time. When observations of stained sections of embryos showed that after 11 hours germination could be found, but after 15 hours of germination. About thirty 11 hr. embryos were found while of thirty 15 hr. embryos none of dividing cells. During the course of nine embryos of each set were prepared in the absence of division. When the supply was found that the 1949 seed (of the same variety) germinated slowly. After checking the slides it was necessary to use a 16 hr. germinating embryo for cell division. A 12 hr. germinating embryo was used for the two conditions. In subsequent experiments embryos were used for the two conditions. In subsequent experiments embryos were checked and failed to divide. Eighty-five 15 and 16 hr. embryos were used for the two conditions.

dinitro-o-cresol (DNC) to inhibit cell

used in the method outlined in A, the purpose was to obtain a group of seedlings where the order to contrast their behavior with the normal. Clowes and Krahel (6) working with 2,4-dinitro-o-cresol (DNC) to inhibit cell division.

7.

Beck and Russell (2) developed a technique for using this agent to decrease mitosis in corn. They immersed the roots of seedlings in a $10^{-5}M$ solution of the DNC for 19 hours at temperatures of 20 to 25° C. and found a noticeable decrease in the amount of cell division. In the experiments described below this technique was used at 20° C.; 20 slides, checked against controls soaked in water under the same conditions, showed a 70% decrease in the number of observable mitoses. In order to provide similar conditions in these experiments, a group of seedlings with roots about 10 mm. long (30 hrs. germination) was divided into two parts. The roots of those in group (a) were immersed in a $10^{-5}M$ solution of DNC for 19 hours at 20° C. while in group (b) the roots were kept in distilled water at the same temperature. Roots in (a) were then carefully washed and all the roots placed in the beakers of sphagnum moss. While measurements at this time (see graph IV, 50 hrs.) showed that DNC inhibited increase in root length, later measurements indicated that the DNC-treated roots quickly recovered and their growth soon paralleled that of the controls. At the end of the 19 hour soaking period roots from both groups were removed, and stained sections of seven roots for each experiment continued to show a 70% reduction in mitosis in the DNC treated roots (a) when compared with the controls (b). This method made it possible to use 2 groups of seedlings germinated for the same length of time and therefore having attained an approximately equal amount of differentiation and hydration, but having a significant difference in the percentage of dividing cells.

Criteria for measurement of degree of injury

Certain criteria were established for recognizing the effects of the

agents used. A seedling whose primary root did not grow more than 8 mm. over a 72 hour period was considered to be dead. Because of the technique employed it was possible to keep an accurate record of primary root length for each individual. One of the more delicate criteria for recognizing injury to a root system by X-rays was described by Martius (14) who used Vicia faba seedlings. He found that delay in the time of appearance of laterals, or the complete inhibition of laterals, was proportional to the dosage of X-rays they received. Russell found this to be true with corn when treated with X-rays (19) and also when corn was exposed to Neutrons (18) and HN_2 (20). Therefore there were 3 criteria for comparison of injury:

1. The percentage of primary roots dying.
2. The percentage of roots showing laterals at the end of the experiment.
3. The time of appearance of the laterals in cases where all groups had laterals at the end of the experiment.

Graphs

The graphical representation of root growth was accomplished by plotting the averages of the length of those dying, and the averages of the living and controls, as a function of time. The ordinate represents the length of the primary root in millimeters and the abscissa the time in hours from the start of germination. The curves representing the growth of the roots where cell division was present at the time of treatment are represented by a solid line, those of the roots where cell division was absent or decreased, by a broken line. The points of measurement are marked by a

the latter group suffered much. When the laterals from HN_2 had corresponding averages are 74 or absence of cell division on laterals.

A comparison of the data and IV confirms the results of I and II. When X-rays were used growing in the 100% normal group; the lateral roots also the other hand, when cell division HN_2 treatment (Table IV) there this group and in the group with

Data from one experiment form: for instance the figures. The key has already been explained. are basically the same but the the two conditions of division ($P < .01$). While 97% of the at the time of radiation died, division were killed. Comparison similarity of the curves produced the percentage dead in this experiment the same for the 16 and 12 hr.

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the latter group suffered much less from the effects of radiation.
When the laterals from HN_2 material in Table II are studied, the
corresponding averages are $74\% \pm 3.4$ and $70\% \pm 3.5$, showing that the presence
or absence of cell division makes no difference in the effect of HN_2
on laterals.

A comparison of the data obtained by using Method B in Tables III
and IV confirms the results obtained by the use of Method A in Tables
I and II. When X-rays were used (Table III), twice as many roots stopped
growing in the 100% normal mitosis group as stopped in the 30% mitosis
group; the lateral roots also showed more damage in the 100% mitotic group. On
the other hand, when cell division was partially stopped with DNC before
 HN_2 treatment (Table IV) there was no difference between HN_2 injury in
this group and in the group which did not receive the DNC.

Data from one experiment in each table are presented in graphical
form: for instance the figures in Table I, Exp. 3 are shown in Graph I.
The key has already been explained. The growth curves for those dying
are basically the same but the difference in the percentage dead under
the two conditions of division and non-division is strikingly significant
($P = < .01$). While 97% of the 16 hr. embryos with cell division present
at the time of radiation died, only 23% of the 12 hr. without cell
division were killed. Comparing Graph I with Graph II shows the great
similarity of the curves produced by the action of the two agents but
the percentage dead in this experiment where HN_2 was used is significantly
the same for the 16 and 12 hr. groups. The HN_2 to all appearances had

TABLE I

X-ray Sensitivity in Relation to Cell Division (Method A)

Exp't #	X-ray dose	Cell Division Present				Cell Division Absent			
		1 Germ. time	2 No. of roots	3 % dead	4 % with laterals	5 Germ. time	6 No. of roots	7 % dead	8 % with laterals
1	4000 r	15 hr.	13	38	34	11 hr.	15	20	75
2	3500 r	15 hr.	14	43	100	11 hr.	14	21	100
3*	4000 r	16 hr.	29	97	21	12 hr.	30	20	94
4	4000 r	16 hr.	25	84	48	12 hr.	29	41	94

Total 81 Av. 65 ± 5.3 51 ± 5.5 Total 88 Av. 25 ± 4.7 91 ± 3.0

* Experiment shown in Graph I

TABLE II

HN₂ Sensitivity in Relation to Cell Division (Method A)

Exp't #	HN ₂ dose 5.02 x 10 ⁻⁵	Cell Division Present				Cell Division Absent			
		1 Germ. time	2 No. of roots	3 % dead	4 % with laterals	5 Germ. time	6 No. of roots	7 % dead	8 % with laterals
1	30 min.	15 hr.	29	45	69	11 hr.	28	75	47
2	30 min.	15 hr.	36	55	80	11 hr.	33	40	90
3*	30 min.	15 hr.	33	55	55	11 hr.	30	56	55
4	30 min.	16 hr.	32	62	94	12 hr.	72	45	86

Total 165 Av. 54 ± 3.9 74 ± 3.4 Total 163 Av. 55 ± 3.4 70 ± 3.5

* Experiment shown in Graph II

X-ray Sensitivity in

Exp't #	Treatment
1*	a) DNC + 17 b) H ₂ O + 17
2	a) DNC + 17 b) H ₂ O + 17

* Experiment shown

HN₂ Sensitivity in R

Exp't #	Treatment 5.2 x 10 ⁻⁶ HN ₂
1	a) DNC + 20 ml b) H ₂ O + 20 ml
2*	a) DNC + 15 ml b) H ₂ O + 15 ml

* Experiment shown in

** In these tables 100

TABLE I

Sensitivity to Cell Division (Method A)

Present		Cell Division Absent			
Exp't	4 % with laterals	5 Germ. time	6 No. of roots	7 % dead	8 % with laterals
1	34	11 hr.	15	20	75
2	100	11 hr.	14	21	100
3	21	12 hr.	30	20	94
4	48	12 hr.	29	41	94

5.3 51±5.5 Total 86 Av. 25±4.7 91±3.0

TABLE II

Sensitivity to Cell Division (Method A)

Present		Cell Division Absent			
Exp't	4 % with laterals	5 Germ. time	6 No. of roots	7 % dead	8 % with laterals
1	69	11 hr.	28	75	47
2	80	11 hr.	33	40	90
3	55	11 hr.	30	56	55
4	94	12 hr.	72	45	86

5.3 74±3.4 Total 163 Av. 55±3.4 70±3.5

TABLE III

X-ray Sensitivity in Relation to Cell Division (Method B) 48 hr. Germination

Exp't	Treatment	Mitosis**	No. of roots	% roots growth stopped	% roots with laterals	Time of appearance of laterals
1*	a) DNC + 1750 r	30%	43	32±7.1	100	b delayed more than a
	b) H ₂ O + 1750 r	100%	39	75±6.9	59	
2	a) DNC + 1700 r	30%	37	37±7.8	97	b delayed more than a
	b) H ₂ O + 1700 r	100%	34	70±7.8	85	

Total 154

* Experiment shown in Graph III

TABLE IV

HN₂ Sensitivity in Relation to Cell Division (Method B) 48 hr. Germination

Exp't	Treatment 5.2x10 ⁻⁶ HN ₂	Mitosis**	No. of roots	% roots growth stopped	% roots with laterals	Time of appearance of laterals
1	a) DNC + 20 min. HN ₂	30%	28	71±8.5	100	all at about the same time
	b) H ₂ O + 20 min. HN ₂	100%	14	71±12.1	100	
2*	a) DNC + 15 min. HN ₂	30%	34	50±8.5	100	all at about the same time
	b) H ₂ O + 15 min. HN ₂	100%	26	53±9.7	100	

Total 102

* Experiment shown in Graph IV

** In these tables 100% mitosis means the normal mitotic index for a given tissue

the same effect regardless of the state of cell division. Graphs III and IV where Method B was used demonstrate this same fact. The curves in both graphs are similar, but in Graph III, X-ray treatment of the roots with decreased mitosis showed only $32\% \pm 7.1$ dying while $75\% \pm 6.9$ of those with normal division were killed. Graph IV shows not only a similarity of the growth curves but also almost identical percentages dying, $53\% \pm 9.7$ and $50\% \pm 8.5$, when both groups were exposed to HN_2 .

DISCUSSION

Certain aspects of the methods used in these experiments can now be more fully discussed in the light of the results obtained. It may be noted that the dosages of both X-rays and HN_2 required to give the desired effect were considerably greater in the case of Method A than Method B. In Method A the difference in the degree of hydration or in the permeability of the pericarp might have necessitated the stronger treatment. Henshaw and Francis working on Triticum vulgare (12) showed that an increase in hydration increased sensitivity to X-rays, and Miller and McElroy working with Neurospora (15) suggested the possibility of a similar relationship between hydration and HN_2 effects. The difference in the extent of hydration between 16 and 48 hour embryos might be sufficient to cause the difference in sensitivity (especially to radiation) between these two stages of development. The permeability of the pericarp would be an important factor in the case of HN_2 , since in the 16 hour embryos the seed coat is still intact while the 48 hour seedlings have roots 10 to 20 mm. long.

The relation between Method B. However, there considered in the use of a found that this agent inhibited and that it was a respiratory Smith (11) state that an increase in sensitivity to radiation in present study. If there was an increase in respiration it is sensitivity caused by the decrease by DNC to decrease cell division.

As has already been demonstrated inhibit mitosis by immersing in 25°C . for 19 hrs. In this at 30°C . but the treatment of mitotic activity of the organism time for the fact that mitosis

A comparison of Tables I and II shows a difference in the effect of treatment on the DNC treated and control. There is a much greater contrast between the cell division in the 12 hr. exposure in Method B where the DNC treated

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DISCUSSION

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of development. The permeability of the pericarp
ster in the case of HN_2 , since in the 16 hour
still intact while the 48 hour seedlings have

The relation between age and penetrability was kept constant in
Method B. However, there are undoubtedly many biological factors to be
considered in the use of such a chemical as DNC. Clowes and Krah1 (6)
found that this agent inhibited mitosis reversibly in the sea urchin egg
and that it was a respiratory stimulator. Tahmisian (22) and Hayden and
Smith (11) state that an increase in respiratory rate causes an increase
in sensitivity to radiation but there is no evidence for this in the
present study. If there were an increase in sensitivity due to the
increase in respiration it might have been obscured by the decrease in
sensitivity caused by the decreased cell division. The roots treated
by DNC to decrease cell division showed a decrease in X-ray sensitivity.

As has already been described, Russell and Beek (2) used DNC to
inhibit mitosis by immersing the roots in a 10^{-5}M solution at 20 to
25° C. for 19 hrs. In this work the treatment with DNC was also tried
at 30° C. but the treatment at this temperature had no effect on the
mitotic activity of the organism. There is no explanation at the present
time for the fact that mitosis was decreased at 20° C. but not at 30° C.

A comparison of Tables I and III show that there is a more pronounced
difference in the effect of the X-ray on the 12 and 16 hr. embryos than
on the DNC treated and controls. This might be expected since there is
a much greater contrast between the two conditions in Method A of no
cell division in the 12 hr. and active cell division in the 16 hr. than
in Method B where the DNC treatment merely reduced mitosis.

The similarities of the effects of X-rays and HN_2 have been pointed out frequently in the literature. Auerbach (1) found HN_2 could produce approximately the same mutation rate in Drosophila as did X-rays. Bodenstein and Kondritzer (3) pointed out likenesses in the effects of radiation and HN_2 on the nucleic acids during embryonic amphibian development. Dustin (7) brings out the similarity of the two agents in the therapy of malignant growths. Chamutin and Cjessing (5) found that HN_2 caused a decrease in the viscosity of solutions of thymonucleohistone while Errera (8) observed similar changes after irradiation with X-rays. Similarly the action of HN_2 was specifically linked to mitotic activity by Gillette and Bodenstein (9). They exposed amphibian embryos to HN_2 and found that certain organs were affected more than others, presumably because they contained more mitotically active cells.

The results obtained with X-rays in these experiments on corn seem to confirm the generally accepted views (Henshaw and Francis, 12; Mottram, 15) relating the effect of radiation to mitotic activity. Results with the HN_2 on the same organism in the same condition as those which were radiated, failed to show any difference in effect which could be related to mitotic activity. It appears to differ greatly from X-rays in this respect. Strong (21) in a discussion of the induction of mutations makes the statement that nitrogen mustard seemed to affect cells even before there were any evidences of cell division but he gives no experimental data. This would seem to agree with our results.

The data obtained from use of corn seedlings as test material indicate that tissue containing a certain amount of time of radiation is significant, particularly with little or no cell division. However, if similar tissue is used, it appears to be no selective for dividing cells or non-dividing cells. This conforms with the generally accepted view that cells are more sensitive to the lethal action of radiation. On the other hand, HN_2 is found to have a more significant action on regions containing dividing cells. However, in the case of corn, the effect is more significant than tissue containing non-dividing cells.

This work was made possible through a grant awarded under contract EC 001-1-1 to the U.S. Atomic Energy Commission.

The author wishes to thank the staff of the Delaware Hospital for their kindness in making available the facilities of the Delaware Hospital.

The nitrogen mustard was supplied by the Army Chemical Center, Maryland.

s of X-rays and HN_2 have been pointed out. Auerbach (1) found HN_2 could produce effects in Drosophila as did X-rays. He pointed out likenesses in the effects of X-rays and HN_2 during embryonic amphibian development. The similarity of the two agents in the effects of HN_2 and Cjessing (5) found that HN_2 and solutions of thymonucleohistone induced mitotic changes after irradiation with X-rays. HN_2 is specifically linked to mitotic activity. HN_2 exposed amphibian embryos to HN_2 affected more than others, presumably the most actively active cells.

In these experiments on corn seen in Henshaw and Francis, 12; radiation to mitotic activity. HN_2 in the same condition as X-rays show any difference in effect which It appears to differ greatly from X-rays in a discussion of the induction of mitosis. Nitrogen mustard seemed to affect the rates of cell division but he gives no reason to agree with our results.

CONCLUSIONS

The data obtained from this series of experiments are based on the use of corn seedlings as test material. The results obtained indicate that tissue containing a considerable number of dividing cells at the time of radiation is significantly more sensitive to X-rays than tissue with little or no cell division going on at the time of exposure. However, if similar tissue is treated with a solution of HN_2 , there appears to be no selective lethal action of HN_2 on either actively dividing cells or non-dividing cells. The results with radiation conform with the generally accepted conclusion that most dividing cells are more sensitive to the lethal action of radiation than resting cells. On the other hand, HN_2 is frequently described as having a selective action on regions containing actively proliferating tissues (10). However, in the case of corn, HN_2 failed to affect such tissue any more significantly than tissue containing few or no dividing cells.

ACKNOWLEDGEMENTS

This work was made possible through the assistance of a fellowship awarded under contract N8 onr 78300 through the joint sponsorship of the U.S. Atomic Energy Commission and the Office of Naval Research.

The author wishes to thank Dr. W.W. Lattonus and Dr. J.W. Alden for their kindness in making available the use of the X-ray facilities at Delaware Hospital.

The nitrogen mustard was made available through the courtesy of the Army Chemical Center, Maryland.

The author also extends thanks to Dr. Mary A. Russell for her advice and encouragement throughout the course of this work and also to the other members of the department for their helpful aid and criticism.

- (1) Auerbach, C., Robson, G.
of mutations. Science 10
- (2) Beck, L.V., and Russell,
- (3) Bodenstein, D., and Kondr
on nucleic acids during e
Zool. 107:109-22, 1948.
- (4) Bryson, V. Effects of ni
Bact. 56:423, 1948.
- (5) Chanutin, A., and Cjessing
- (6) Clowes, G.H.A., and Krahls
mechanism involved in dini
Proc. Soc. Exp. Biol. Med.
- (7) Dustin, P. Jr. Mitotic po
growth. Unio Intern. Contr
- (8) Errera, M. Action des radi
cellulaire. Bull. Soc. Chi
- (9) Gillette, R., and Bodenstein
produced in amphibian embryo
Exp. Zool. 103:1-32, 1946.
- (10) Gilman, A., and Phillips, P.S
applications of the B-chloro
40% 1946.
- (11) Hayden, E., and Smith, L. Th
effects of X-rays. Genetics

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helpful aid and criticism.

BIBLIOGRAPHY

- (1) Auerbach, C., Robson, G.M., and Carr, J.G. The chemical production of mutations. *Science* 105:243-7, 1947.
- (2) Beck, L.V., and Russell, M.A. Unpublished Report.
- (3) Bodenstein, D., and Kondritzer, A.A. The effect of nitrogen mustard on nucleic acids during embryonic amphibian development. *J. Exp. Zool.* 107:109-22, 1948.
- (4) Bryson, V. Effects of nitrogen mustard on *Escherichia coli*. *J. Bact.* 56:423, 1948.
- (5) Chanutin, A., and Cjessing, E. *Cancer Research* 6:496-9 1946.
- (6) Clowes, G.H.A., and Krah1, M.E. Observations on cellular oxidative mechanism involved in dinitrophenol stimulation of respiration. *Proc. Soc. Exp. Biol. Med.* 32:226-38 1934.
- (7) Dustin, P. Jr. Mitotic poisons in the chemotherapy of malignant growth. *Unio Intern. Contra Cancrum, Acta* 6:466-77, 1949.
- (8) Errera, M. Action des radiations sue les nucleoproteines du noyau cellulaire. *Bull. Soc. Chim. Biol.* 28:472-7, 1946.
- (9) Gillette, R., and Bodenstein, D. Specific developmental inhibitions produced in amphibian embryos by a nitrogen mustard compound. *J. Exp. Zool.* 103:1-32, 1946.
- (10) Gilman, A., and Philips, F.S. The biological actions and therapeutic applications of the B-chloroethyl amines and sulfides. *Science* 103: 409 1946.
- (11) Hayden, B., and Smith, L. The relation of atmosphere to biological effects of X-rays. *Genetics* 34:26-43, 1949.

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Growth of primary roots followed

Key:

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- - - 12 hour embryos (mito

■ Controls

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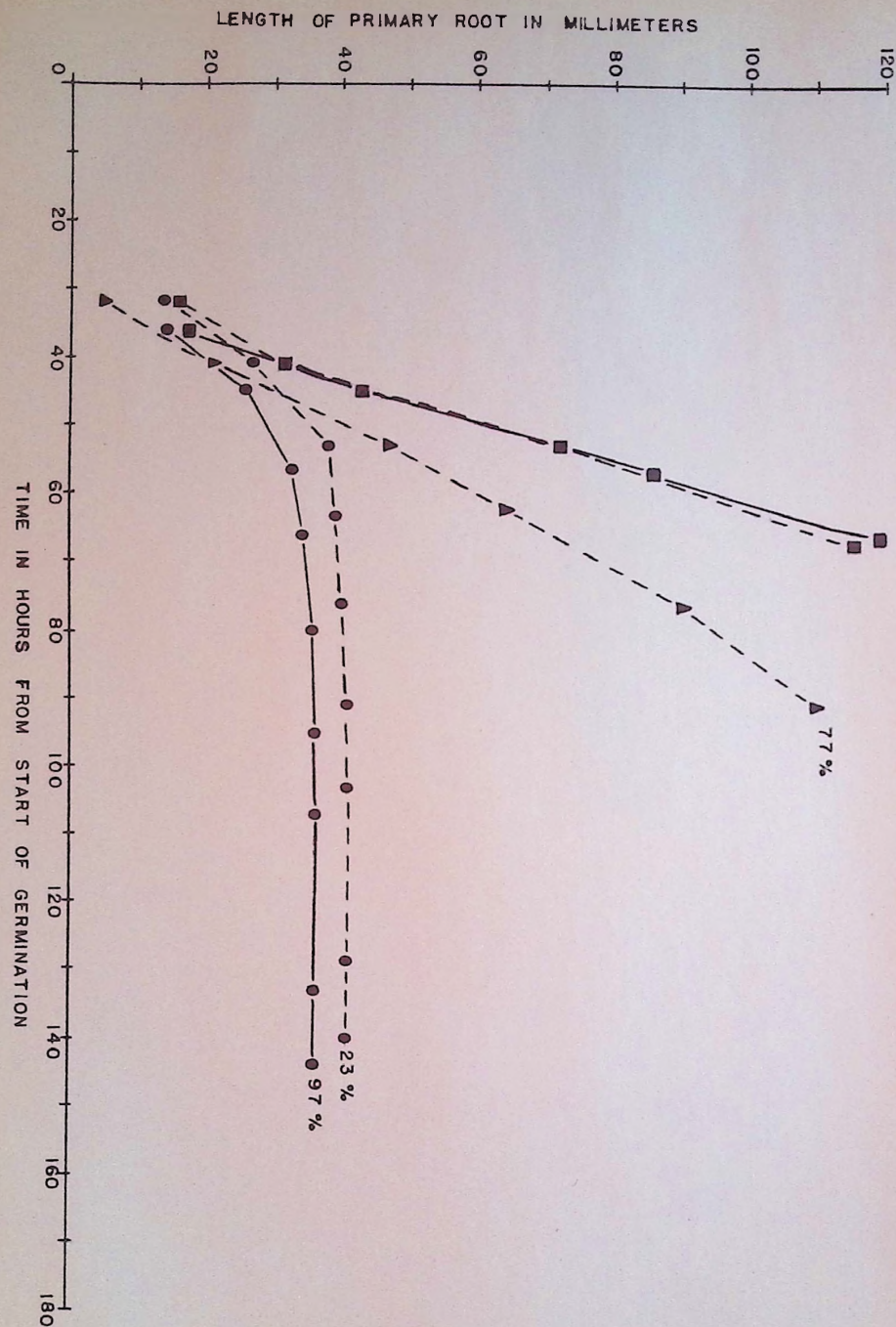
GRAPH I

(Table I)

Growth of primary roots following x-radiation of embryos with 4000 r. *et* 3

Key:

- 16 hour embryos (mitosis present)
- - 12 hour embryos (mitosis absent)
- Controls
- ▲ Living after radiation
- Dead after radiation



Growth of primary roots

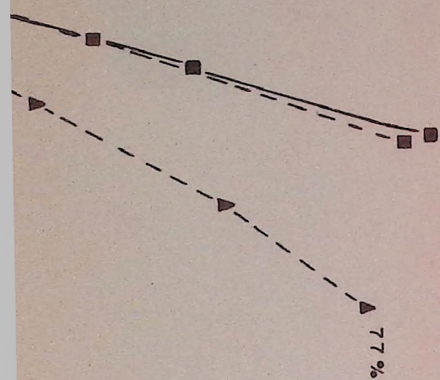
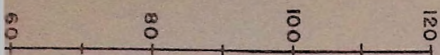
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- - 12 hour embryo
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- Dead after HN_2

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ROOT IN MILLIMETERS



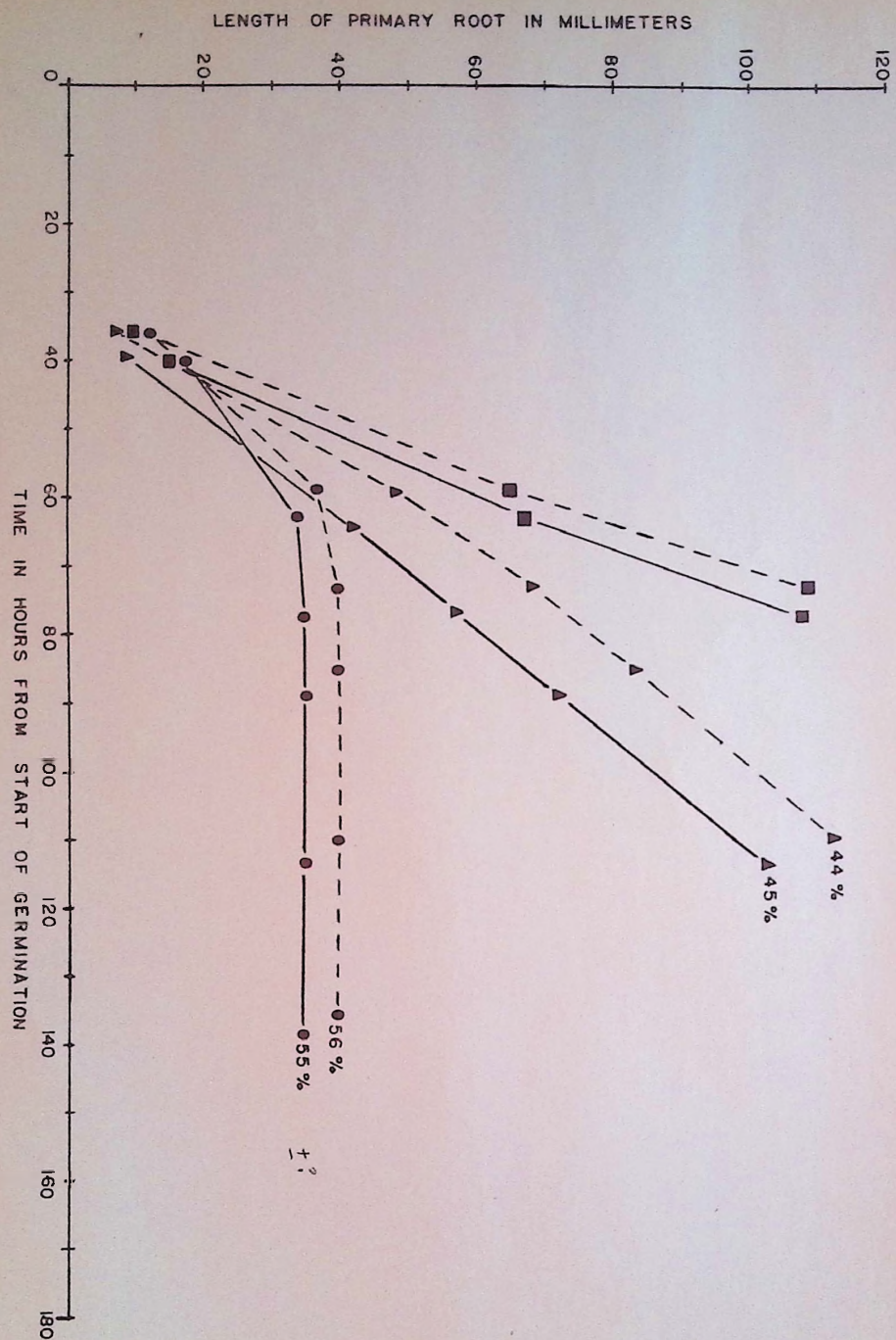
GRAPH II

(Table II)

Growth of primary roots following exposure of embryos to HN_2 .

Key:

- 16 hour embryos (mitosis present)
- - 12 hour embryos (mitosis absent)
- Controls
- ▲ Living after HN_2 treatment
- Dead after HN_2 treatment



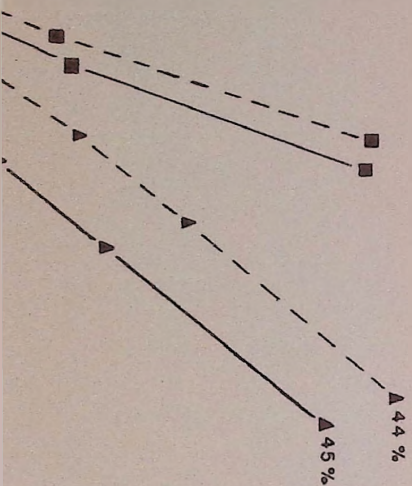
Growth of DNC treated pr

Key:

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- - - DNC treatment
- Controls
- ▲ Living after r
- Dead after rad

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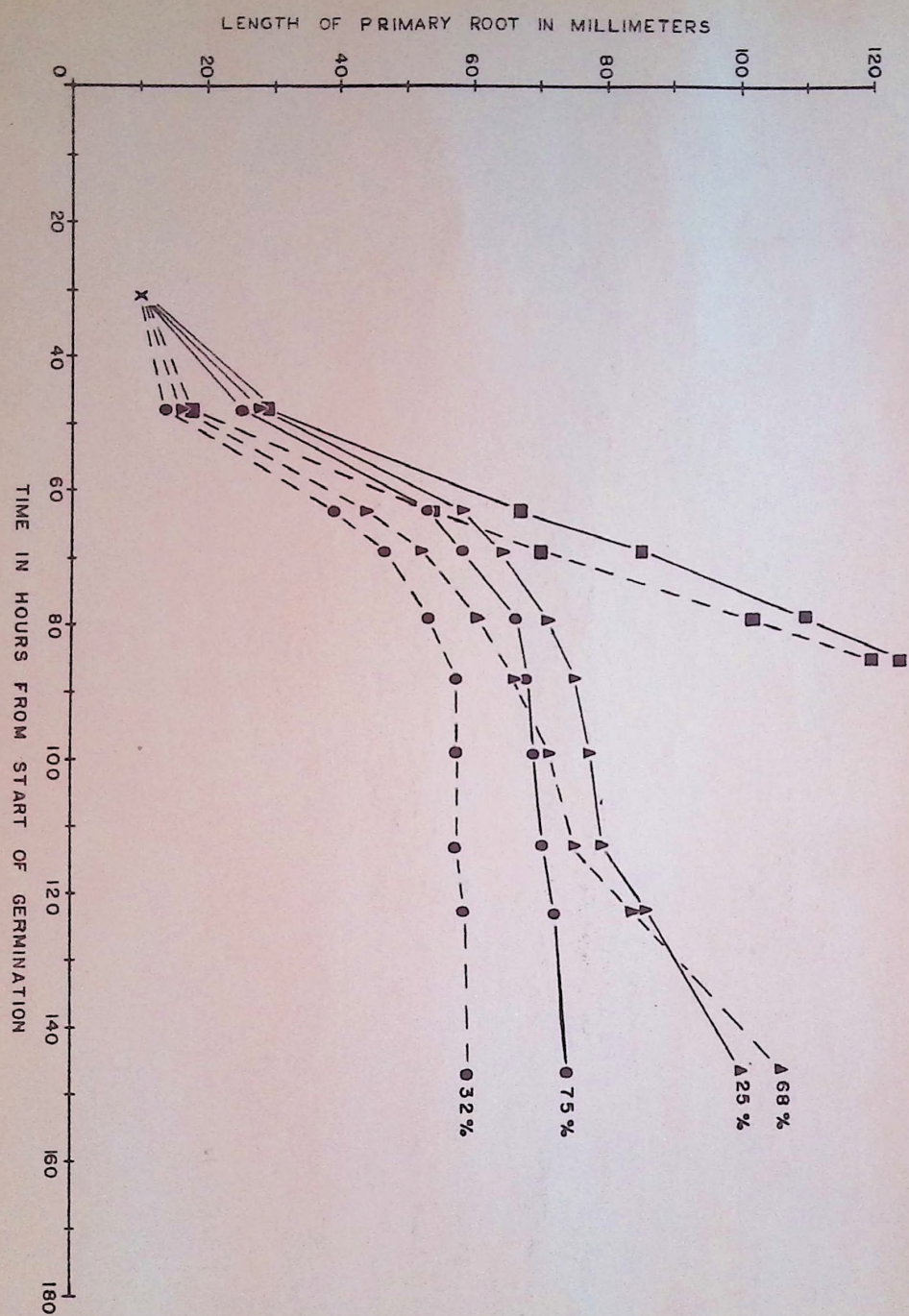
GRAPH III

(Table III)

Growth of DNC treated primary roots following x-radiation with 1750 r.

Key:

- H₂O treatment (mitosis present)
- - - DNC treatment (mitosis reduced)
- Controls
- ▲ Living after radiation
- Dead after radiation



Growth of DNC treat

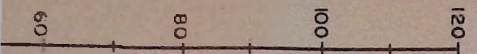
Key:

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- - - DNC treat
- Controls
- ▲ Living a
- Dead after

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BY ROOT IN MILLIMETERS



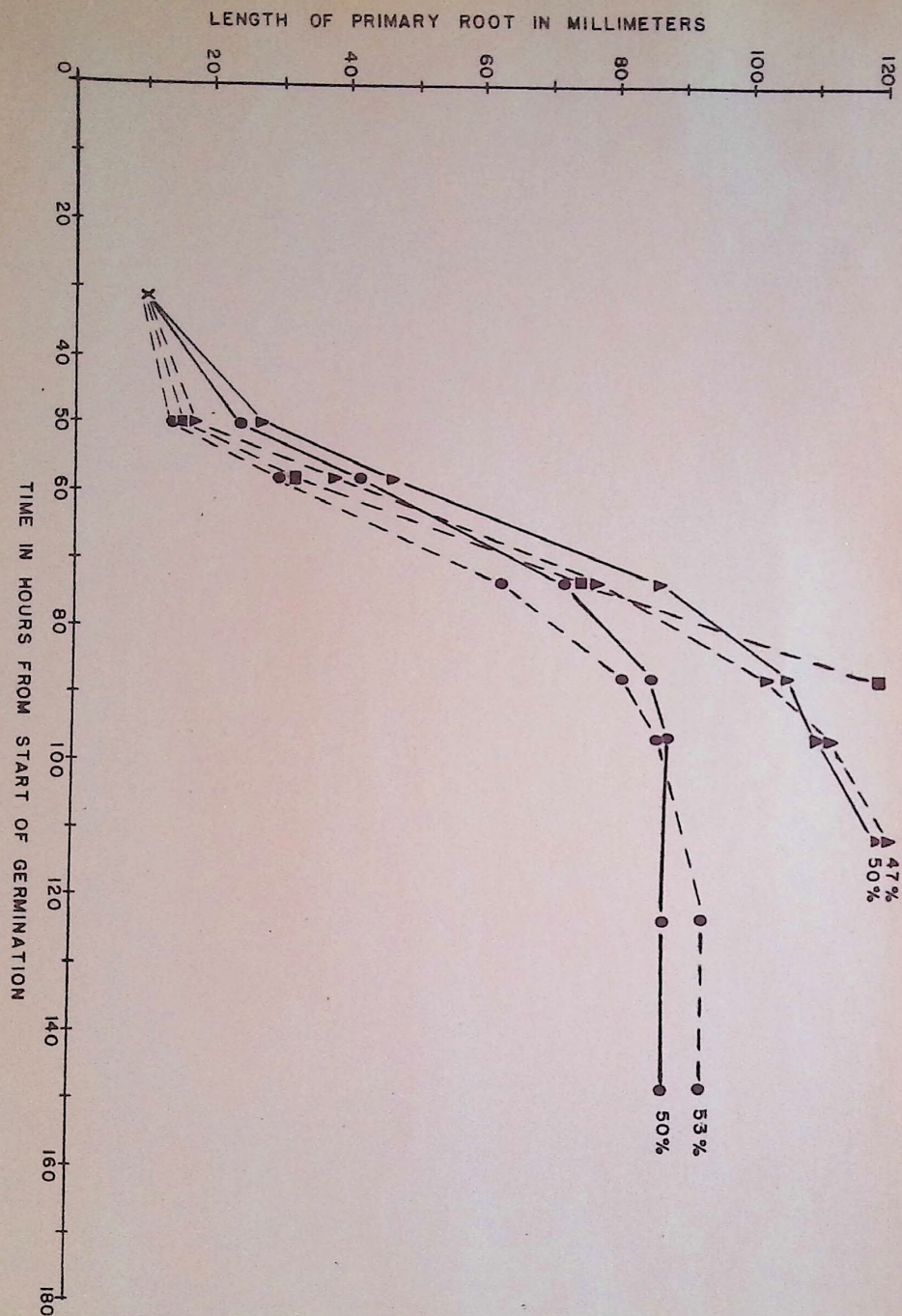
GRAPH IV

(Table IV)

Growth of DNC treated primary roots following exposure to HN_2 .

Key:

- H_2O treatment (mitosis present)
- - - DNC treatment (mitosis absent)
- Controls
- ▲ Living after HN_2 treatment
- Dead after HN_2 treatment



Relation of Mitotic Act and Nitrogen Mus Growth of

MARY A. RUSSELL

(Department of Biological Science)

In their review of the early research on mustard compounds, Gilman and Philips (3) list several striking similarities between the biological effects of these chemicals and radiation. They report that "Actively proliferating cells are selectively vulnerable to the cytotoxic action of the mustard." There is general agreement that, in most cases, dividing cells are more sensitive to radiation than resting cells, but recent literature indicates a conflict of opinion as to whether dividing cells are necessarily more sensitive to the effects of mustard. Friedenwald and Buschke (2) have discussed the difficulty of the problem. They point out that while some tissues with many dividing cells, such as bone marrow and intestinal mucosa, are very sensitive to mustard, a tissue like liver with active cell division stimulated by partial hepatectomy, is comparatively insensitive.

The purpose of this investigation is to compare the sensitivity of tissue containing many dividing cells with the same kind of tissue in which little or no cell division is present at the time of exposure to either x-rays or nitrogen mustard. The root of the corn seedling has been found to be a satisfactory organism for studying the effects of both x-rays (5) and nitrogen mustard (6). It is particularly suitable for this problem, because tissue can be obtained with no cell division or with varying percentages of dividing cells by controlling the length of the germination period or by chemical treatment.

MATERIALS AND METHODS

Corn seed of the "U.S. 13" strain was treated for 5 minutes in a 0.05 per cent mercurophen solution to prevent the growth of mold. After soaking

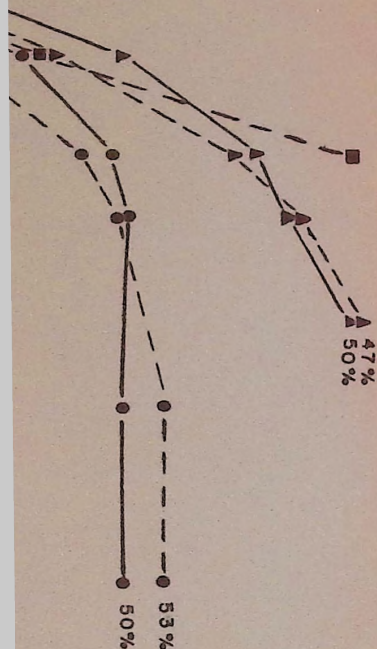
* These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Delaware (NR 164-858).

† Present address: Botany Department, University of Pennsylvania, Philadelphia, Pa.

Received for publication March 16, 1951.

MILLIMETERS

80 100 120



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CANCER RESEARCH
Vol. 11, No. 9, pp. 687-93, September 1951
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Relation of Mitotic Activity to the Effects of X-Rays and Nitrogen Mustard as Indicated by the Growth of Corn Seedlings*

MARY A. RUSSELL AND FRANCIS J. MICHELINI†

(Department of Biological Sciences, University of Delaware, Newark, Del.)

In their review of the early research on mustard compounds, Gilman and Philips (3) list several striking similarities between the biological effects of these chemicals and radiation. They report that "Actively proliferating cells are selectively vulnerable to the cytotoxic action of the mustards." There is general agreement that, in most cases, dividing cells are more sensitive to radiation than resting cells, but recent literature indicates a conflict of opinion as to whether dividing cells are necessarily more sensitive to the effects of mustards. Friedenwald and Buschke (2) have discussed the difficulty of the problem. They point out that while some tissues with many dividing cells, such as bone marrow and intestinal mucosa, are very sensitive to mustard, a tissue like liver, with active cell division stimulated by partial hepatectomy, is comparatively insensitive.

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MATERIALS AND METHODS

Corn seed of the "U.S. 13" strain was treated for 5 minutes in a 0.05 per cent mercuriophen solution to prevent the growth of mold. After soaking

in water for 1 hour, the seed was allowed to germinate at 30° C. Tall 600-ml. beakers were lined with filter paper, then loosely filled with moist sphagnum moss (buffered to maintain a pH of about 5), covered with petri dish lids, and autoclaved at 15 lb. pressure for 30 minutes. After the desired period of germination, the seedlings were exposed to x-rays or to a solution of methylbis(β-chloroethyl)amine hydrochloride, designated as HN2. After treatment, about fifteen seedlings were placed in each beaker so that the roots grew down between the paper and the glass. The beakers were incubated at 30° C. A mark was placed on every beaker to indicate the position of the first seedling, so that a record could be kept of each individual. The length of the primary roots was generally measured twice daily, and observations were made at more frequent intervals to determine the time at which the first lateral roots appeared. If the primary roots reached the bottom of the beaker (about 120 mm.), they were classed as survivors, and roots whose length remained constant for 3 days were recorded as having stopped growing.

The x-ray machine used in these experiments was operated at 120 kv. and 10 ma with a 1.5-mm. aluminum filter. The output intensity was 72 r/min. During radiation the seeds were placed with embryo side up and covered with cellophane to prevent dehydration.

The HN2 used in these investigations was furnished by the Army Chemical Center, Maryland. A fresh solution was prepared just before each experiment, with distilled water of about pH 5.7, unless otherwise stated. A temperature of 28°-30° C. was maintained during exposure of the seedlings to HN2, and they were washed for 5 minutes in running tap water to remove all traces of the chemical.

Two methods were used to obtain tissue with little or no cell division for comparison with similar tissue containing a higher percentage of dividing cells.

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ROOT IN MILLIMETERS



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MATERIALS AND METHODS

Corn seed of the "U.S. 13" strain was treated for 5 minutes in a 0.05 per cent mercurophen solution to prevent the growth of mold. After soaking

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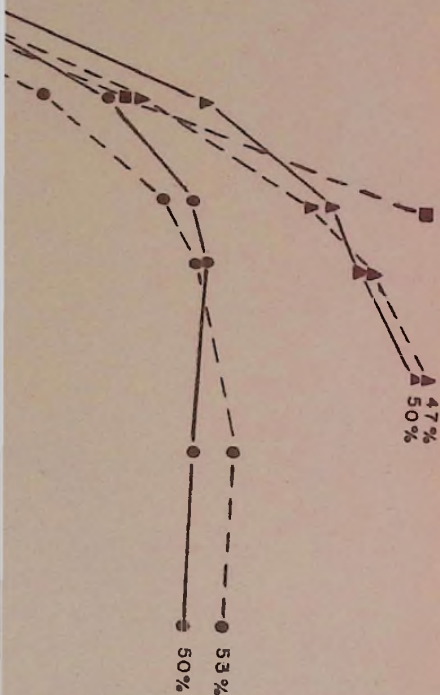
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The HN2 used in these investigations was furnished by the Army Chemical Center, Maryland. A fresh solution was prepared just before each experiment, with distilled water of about pH 5.7, unless otherwise stated. A temperature of 28°-30° C. was maintained during exposure of the seedlings to HN2, and they were washed for 5 minutes in running tap water to remove all traces of the chemical.

Two methods were used to obtain tissue with little or no cell division for comparison with similar tissue containing a higher percentage of dividing cells.

ROOT IN MILLIMETERS

60 80 100 120



METHOD A

Use of embryos germinated for different periods of time.—Stained sections of the radicle tips of embryos germinated for 12 hours showed no mitotic figures, but after 15 or 16 hours of germination, about 15 per cent of the cells in the meristematic region of the tip were in some stage of mitosis. Therefore, 15 or 16 hours were allowed for germination when tissue with cell division was desired, and 11 or 12 hours when the experiment called for tissue without cell division. These two germination periods were chosen because it was desirable to use tissue in as nearly the same physiological condition as possible, but at the same time to make certain that the 4 hours' difference would divide the two groups into one with cell division and one without cell division. In preparing for these experiments, one set of seeds was started germinating 4 hours before the other, so that both sets would be ready for exposure to either x-rays or HN2 at the same time. In the HN2 experiments, controls were placed in distilled water during the time when the seedlings were soaked in the HN2 solution.

METHOD B

Use of seedlings treated with 3,5-dinitro-o-cresol (DNC), to inhibit cell division for comparison with normal seedlings.—Clowes and Krahl (1) reported that DNC was a reversible mitotic inhibitor when its action was tested on sea urchin eggs. Beck and Russell¹ later found that corn roots would resume normal growth after cell division was decreased in the root tip by means of exposure to DNC. Seedlings with roots about 10 mm. long were divided into two groups, as follows: the roots of group 1 were immersed in a 10^{-5} M solution of DNC for 19 hours at 20° C. and washed in running water for 5 minutes; the roots in group 2 were kept in distilled water for the same time at the same temperature. Both groups were then ready for exposure to either radiation or HN2. During the soaking period, the DNC-treated roots showed no appreciable growth, whereas the controls grew normally. Charts 2 and 3 show that the DNC-treated roots quickly recovered, and their growth soon paralleled that of the controls. It was noted in sections prepared from root tips fixed at the end of the 19-hour soaking period that about 22 per cent of the total cells in the meristematic region were dividing in group 1, while in the normal group (2) about 38 per cent were in some stage of division. Part of each group was kept untreated as controls.

¹ L. V. Beck and M. A. Russell, unpublished report, 1934.

RESULTS

Table 1 shows that, in every experiment, the percentage of roots which stopped growing was always higher in the group of seedlings with cell division present at the time of radiation. When the averages from all the experiments are compared, the difference is very striking ($P < 0.001$). A study of the lateral roots showed that the seedlings treated at a time after cell division had started were more sensitive to radiation than the seedlings in which there was no cell division at the time of treatment. In the group in which cell division was present, only 60 per cent of the seedlings produced laterals, while in the second group 97 per cent had laterals. In the first group, the initial appearance of the laterals was delayed longer than in the second group. All these facts indicate that the seedlings in the group with cell division suffered the greater amount of radiation injury.

In Table 2, the behavior of the primary roots after treatment with HN2 shows that there was much less difference in sensitivity to this agent between the group with dividing cells and the group with no cell division at the time of exposure to the HN2 solution. The difference is not significant ($0.01 < P < 0.02$). A study of the lateral roots showed that, although the first of these appeared in both groups at the same time, they were more numerous in the group without cell division, showing these seedlings to be slightly more resistant to HN2.

Chart 1 shows the growth curves of the primary roots which died in two typical experiments using Method A (Table 1, Exp. 3, and Table 2, Exp. 3). The shape of the curve made from data from roots treated with HN2 is very similar to the curve made from the data from the x-rayed roots, and the final length of the primary roots is about the same in both cases.

Chart 2 shows the behavior of the primary roots in a typical experiment (Exp. 3) from Table 3. In each experiment of this series, a larger percentage of roots stopped growing in the normal cell division group than in the reduced cell division group. The difference is highly significant ($P < 0.001$). Again, the laterals appeared later in the former group, and at the end of the experiment only 83 per cent of these seedlings had laterals, as compared to the presence of laterals in 99 per cent of the group with reduced mitotic activity. All this evidence indicates that seedlings with a normal mitotic index were more sensitive to radiation than those in which the number of dividing cells was reduced by DNC treatment.

Chart 3 was constructed on the basis of Exp. 3 in the series recorded in Table 4. This table shows

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Figure 1 shows the growth curves of the primary roots which died in two typical experiments using method A (Table 1, Exp. 3, and Table 2, Exp. 3). The shape of the curve made from data from roots treated with HN2 is very similar to the curve from the data from the x-rayed roots, and the total length of the primary roots is about the same in both cases.

Figure 2 shows the behavior of the primary roots in a typical experiment (Exp. 3) from Table 3. In this experiment of this series, a larger percentage of roots stopped growing in the normal cell division group than in the reduced cell division group. The difference is highly significant ($P < 0.001$). Again, laterals appeared later in the former group, and at the end of the experiment only 83 per cent of the seedlings had laterals, as compared to the percentage of laterals in 99 per cent of the group with normal mitotic activity. All this evidence indicates that seedlings with a normal mitotic index are more sensitive to radiation than those in which the number of dividing cells was reduced by treatment.

Figure 3 was constructed on the basis of Exp. 3 of the series recorded in Table 4. This table shows

RUSSELL AND MICHELINI—*Relation of Mitosis to Effects of X-Rays and a Mustard* 689

that when the two groups of seedlings were treated with identical doses of HN2, the group with a normal mitotic index was more sensitive to radiation than the group with a reduced mitotic index. The use of the two agents, the doses of x-rays and HN2 used in these experiments with 16-hour germinating seedlings, were equivalent to those used in the test material considered to kill the

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RESEARCH REVIEWS

MAY 1950

Nitrogen Mustard and X-Ray Research

by
Mary A. Russell
University of Delaware



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DEPARTMENT OF THE NAVY

WASHINGTON, D. C.

0 and 4,000 r cent of the results of 50 r were recorded in the group greater sensitivity in Table 3 in hydration probable that be attributed

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AND

N ABSENT Treated		Per cent roots stopped growing
No. of roots		
15	20	
14	21	
30	20	
29	41	
28	15	
28	10	
144	22*	

AND

N ABSENT Treated		Per cent roots stopped growing
No. of roots		
28	78	
33	39	
30	56	
72	46	
41	63	
69	91	
273	62†	

RUSSELL AND MICHELINI—*Relation of Mitosis to Effects of X-Rays and a Mustard* 689

when the two groups of seedlings were treated with identical doses of HN₂, the group with a normal rate of cell division was only slightly more sensitive than the group treated with X-rays. The use of the two agents, the doses of x-rays and HN₂ used in these experiments with 16-hour germinated embryos con-

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Nitrogen Mustard and X-Ray Research

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Much attention has been given to mustard compounds since 1941, when Charlotte Auerbach¹ discovered that the compounds could be used to produce mutations similar to those produced by X-rays. Further studies have shown more points of similarity between the action of these compounds and the effects of radiation. One of the more significant is that both agents stop cell division without interfering immediately with cell elongation and differentiation. In addition, both X-rays and mustard compounds produce comparable changes in chromosome structure and behavior as well as other cytological abnormalities. And still another relationship of the supposed similarity between the action of these agents is exemplified by the work of V. Bryson who found that a strain of bacteria (*Escherichia coli*) which was resistant to radiation was also relatively resistant to the action of a mustard. These and other observations may indicate the existence of a common mode of action of these agents on living tissue.

Since the mustards were found to have a pronounced effect on actively growing tissues, it was logical to test their possible therapeutic usefulness in controlling the lawless multiplication of cells in cancer and allied diseases. The most successful results in the application of these compounds have been obtained with conditions involving the abnormal growth of lymphatic tissue. In some cases of Hodgkin's disease and lymphosarcoma, nitrogen mustard has had a palliative effect when used after X-rays had lost their power to control the disease. Occasionally, patients who have failed to respond to radiation treatment initially, have been treated with small amounts of a mustard compound to serve as a sensitizing agent so that subsequent radiation treatment became effective.

These considerations indicate that any further information concerning the action of mustard compounds on living tissue should have both practical and theoretical value. This laboratory is doing research on a project involving a comparison of the effects of mustard compounds and X-rays on the growth and development of the corn seedling. This test medium is an easily obtained source of embryonic tissue, and observations of its behavior may have direct bearing on certain aspects of the cancer problem since cancerous tissue simulates embryonic tissue development.

The compound used in these experiments was methyl-bis (Beta-chloroethyl) amine hydrochloride, furnished through the courtesy of the Army Chemical Center. The accepted symbols for this chemical are

Mary A. Russell received her A. B. from Goucher College and the degree of Ph. D. from the University of Pennsylvania. She was a Research Assistant at the Biochemical Research Foundation from 1928 to 1942. Since 1942 Dr. Russell has been a member of the Department of Biological Sciences at the University of Delaware, Newark, Del.

Treated	Per cent
of roots	stopped
ts growing	
20	
21	
20	
41	
15	
10	
22*	

Treated	Per cent
of roots	stopped
ts growing	
78	
39	
56	
46	
63	
91	
62†	

RESULTS

Figure 1 shows that, in every experiment, the percentage of roots which stopped growing was higher in the group of seedlings with cell division present at the time of radiation. When the results from all the experiments are compared, the difference is very striking ($P < 0.001$). A study of lateral roots showed that the seedlings which divided at a time after cell division had started were more sensitive to radiation than the seedlings in which there was no cell division at the time of exposure. In the group in which cell division was present, only 60 per cent of the seedlings produced lateral roots, while in the second group 97 per cent had. In the first group, the initial appearance of lateral roots was delayed longer than in the second group. All these facts indicate that the seedlings in the group with cell division suffered the amount of radiation injury.

Table 2, the behavior of the primary roots of seedlings treated with HN2 shows that there was no difference in sensitivity to this agent between the group with dividing cells and the group with no cell division at the time of exposure to HN2 solution. The difference is not significant ($0.01 < P < 0.02$). A study of the lateral roots showed that, although the first of these appeared in both groups at the same time, they were more numerous in the group without cell division, and these seedlings to be slightly more resistant to HN2.

Figure 2 shows the growth curves of the primary roots of seedlings which died in two typical experiments using HN2 (Table 1, Exp. 3, and Table 2, Exp. 3). The curve made from data from roots of seedlings with HN2 is very similar to the curve made from the data from the x-rayed roots, and the length of the primary roots is about the same in both cases.

Figure 3 shows the behavior of the primary roots of seedlings in a larger experiment (Exp. 3) from Table 3. In the normal cell division group, a larger percentage of seedlings stopped growing in the normal cell division group than in the reduced cell division group. The difference is highly significant ($P < 0.001$). Again, lateral roots appeared later in the former group. At the end of the experiment only 83 per cent of the seedlings had laterals, as compared to the 99 per cent of the group with normal mitotic activity. All this evidence indicates that seedlings with a normal mitotic index are more sensitive to radiation than those in which the number of dividing cells was reduced by treatment.

The growth curve was constructed on the basis of Exp. 3 and is recorded in Table 4. This table shows

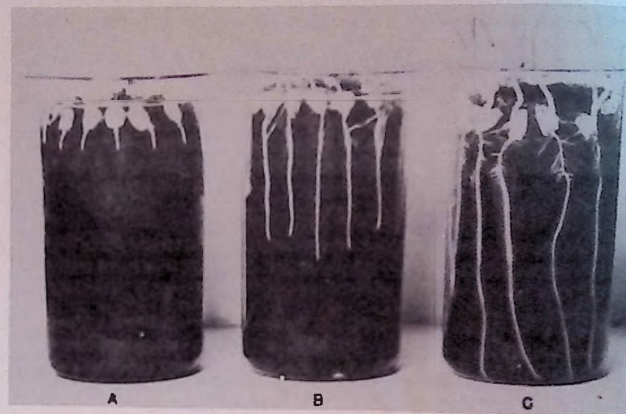


Figure 1. Normal growth of corn seedlings. (a) Appearance when first placed in beakers of moss. (b) After 36 hours, no laterals formed. (c) After three days, laterals are well developed.

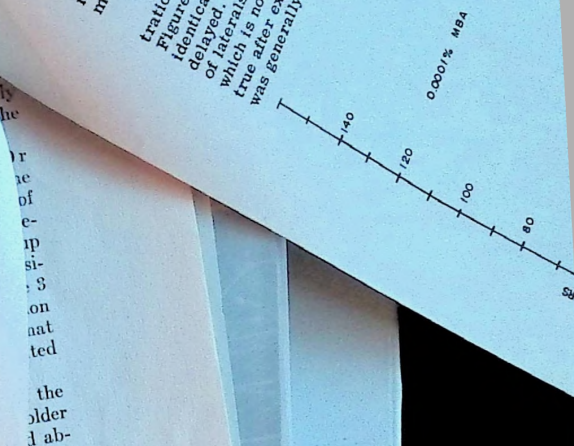
MBA or HN2. Since this compound is water soluble, it is more convenient for test material than other forms. When this work was started, the only guides to techniques were a few papers by Gillette and Bodenstein describing the effects of MBA solutions on amphibian larvae. The importance of using freshly made solutions of MBA was stressed. One experiment, for example, indicated that a solution made up at pH 6.8 was inactive after standing for 45 minutes. An organic chemist advised that if solutions were made with distilled water at approximately pH 5.7, the compound would remain more stable for a longer period of time. A 0.001 percent solution of MBA was prepared in this manner, having an initial pH of 5.3 which changed to pH 4.9 by the end of six hours. At this time seedlings were treated with the solution, and subsequent observations of their growth showed that they were damaged to the same degree as seedlings similarly treated with a fresh solution.

Corn of the "U.S. 13" strain is being used in these experiments. The seed is routinely treated for five minutes with 0.05 percent mercuric solution to kill mold spores. It is then soaked in water for one hour, spread between layers of wet paper towels in germinating dishes and put into an incubator. A temperature of 30°C is used during the entire experiment. After about 30 hours, seedlings with roots of from five to 10 mm length are selected for use. Tall 600 ml beakers are lined with filter paper and loosely packed with moist sphagnum moss. They are covered with petri dishes and sterilized to prevent growth of mold. After immersion in the MBA solution for the required length of time, the seedlings are washed thoroughly with tap water and then placed between the glass and the paper, about 15 plants to each beaker. (See Figure 1.) Thirty seedlings are used in each series. Controls are soaked in distilled water for the same period of time as that used for

Total
* pH of water higher, HN2 solution pH 6.6.
† Weighted averages.

the MBA solutions. The beakers are marked so that their own number, and its individual record is kept. Measurements of the length of the roots are made twice daily, and more frequent observations determine the time of appearance of the lateral roots.

The behavior of the primary roots after exposure to Figure 2. After treatment for five minutes, the growth rate of lateral roots is delayed. This example illustrates the fact that delay in the appearance of laterals indicates some degree of damage to the organism as a whole which is not shown in the behavior of the primary roots. The true after exposure to X-rays. After 15 minutes' exposure, the growth was generally good, but one very sensitive root died. The roots treated



Time (min)	Per cent roots stopped growing
0	20
5	21
4	20
10	41
20	15
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28	22*
144	

AND

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28	78
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RUSSELL AND MICHELINI—*Relation of Mitosis to Effects of X-Rays and a Mustard* 689

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The behavior of the primary roots after exposure to one concentration of MBA (0.0001 percent) for different periods of time is shown in Figure 2. After treatment for five minutes, the growth rate was almost identical with that of the controls, but the lateral roots were slightly delayed. This example illustrates the fact that delay in the appearance of laterals indicates some degree of damage to the organism as a whole which is not shown in the behavior of the primary roots. The same is true after exposure to X-rays. After 15 minutes' exposure, the growth was generally good, but one very sensitive root died. The roots treat-

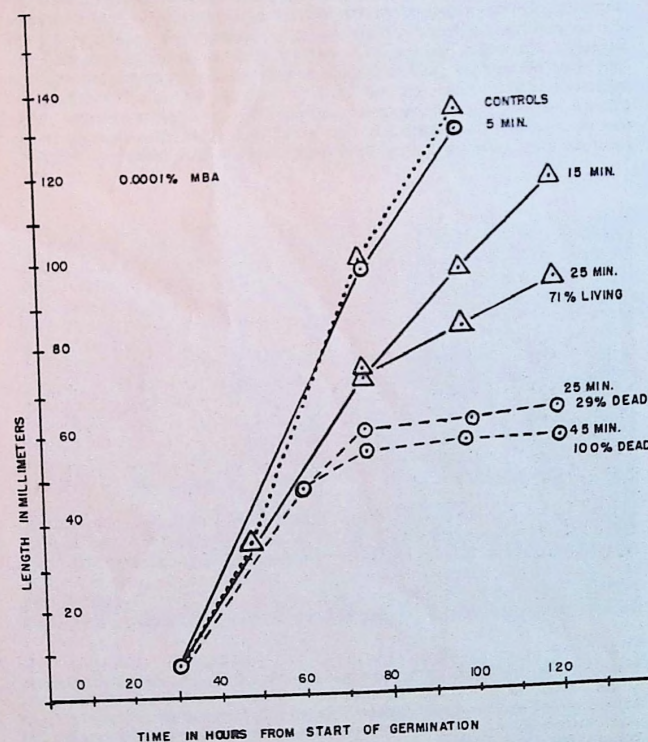


Figure 2. Behavior of primary roots after exposure to 0.0001 percent solution of MBA for different periods of time.

ROOT IN

Figure 1. Normal growth of corn seedlings. (a) First placed in beaker's of moss. (b) After 24 hours, no laterals formed. (c) After three days, laterals are well developed.

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At this time seedlings were treated with the solution, and subsequent observations of their growth showed that they were damaged to the same degree as seedlings similarly treated with a fresh solution.

Corn of the "U.S. 13" strain is being used in these experiments. The seed is routinely treated for five minutes with 0.05 percent mercuric iodine solution to kill mold spores. It is then soaked in water for 24 hours between layers of wet paper towels in germinating trays. After about 20 hours, seedlings with roots of 1-2 cm. are selected for use. Trays 600 ml. beakers are loosely packed with moist sphagnum moss. The solution for the required length of time is selected for use. The solution is then poured into the beaker, and the seedlings are placed in it. The solution is then poured into the beaker, and the seedlings are placed in it. The solution is then poured into the beaker, and the seedlings are placed in it.

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†Weig

RESULTS

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In Table 2, the behavior of the primary roots after treatment with HN2 shows that there was much less difference in sensitivity to this agent between the group with dividing cells and the group with no cell division at the time of exposure to the HN2 solution. The difference is not significant ($0.01 < P < 0.02$). A study of the lateral roots showed that, although the first of these appeared in both groups at the same time, they were more numerous in the group without cell division, showing these seedlings to be slightly more resistant to HN2.

Chart 1 shows the growth curves of the primary roots which died in two typical experiments using Method A (Table 1, Exp. 3, and Table 2, Exp. 3). The shape of the curve made from data from roots treated with HN2 is very similar to the curve made from the data from the x-rayed roots, and the final length of the primary roots is about the same in both cases.

Chart 2 shows the behavior of the primary roots in a typical experiment (Exp. 3) from Table 3. In each experiment of this series, a larger percentage of roots stopped growing in the normal cell division group than in the reduced cell division group. The difference is highly significant ($P < 0.001$). Again, the laterals appeared later in the former group, and at the end of the experiment only 83 per cent of these seedlings had laterals, as compared to the presence of laterals in 99 per cent of the group with reduced mitotic activity. All this evidence indicates that seedlings with a normal mitotic index were more sensitive to radiation than those in which the number of dividing cells was reduced by DNC treatment.

Chart 3 was constructed on the basis of Exp. 3 in the series recorded in Table 4. This table shows

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ed for 25 minutes were divided into two groups, so that one curve could be prepared to show the progress made by those which survived for four days (71 percent), and another curve made for those which died. The latter curve closely parallels the curve obtained from roots exposed for 45 minutes. This period of exposure was found to be lethal to 100 percent of the roots at the concentration of 0.0001 percent MBA. (See Figure 3a, b and c.)

After testing the effect of exposing seedlings to a single concentration of MBA for various time intervals, experiments were set up to test a series of concentrations as shown in Table I.

The results indicate that a solution of 1:1000 inhibited all growth if seedlings were exposed for 37 minutes, but a solution of 1:100,000 had no lethal effect even when exposed for five hours. When the 1:100,000 solution was used, our results were similar to those obtained with the amphibian larvae exposed to the same concentration, in that exposure of five minutes produced nearly as much damage as exposure for 45 minutes. However, we are quite certain that our solution was still active after 45 minutes, and that little more damage appeared because the maximum effect was reached in five minutes at this concentration. Using a large volume of solution made no difference in our results. This was done to test the possibility that all of the active MBA present in the solution may have been used up during the first five minutes. Experi-

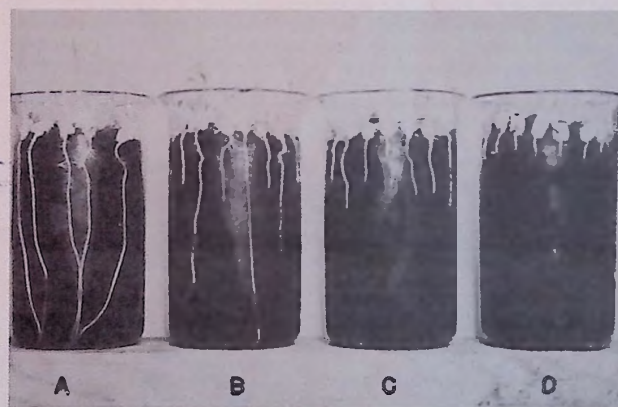


Figure 3. Photo taken three days after treatment of seedlings. (a) Control. (b) Exposed to 0.0001 percent MBA for 25 minutes, showing some roots similar to those in the control beaker (a), while other roots have stopped growing. (c) Exposed to (b) solution for 45 minutes. This dose killed all of the primary roots and prevented formation of lateral roots. (d) Exposed to 0.01 percent CuSO_4 for two hours. Compare with Figure 1(a) and notice that these roots are the same length as they were at the time of exposure.

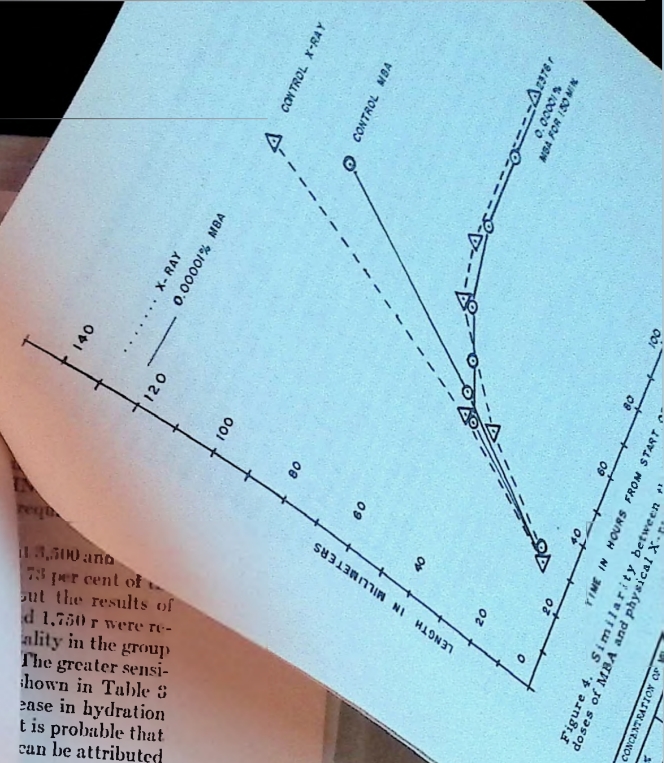


Figure 4. Similarity between the dose of MBA and physical X-ray dose for 100% inhibition of growth.

effectiveness of the to treat the older roots could ab-

TABLE I

Treated	
No. of roots	Per cent roots stopped growing
15	20
14	21
30	20
29	41
28	15
28	10
144	22*

TABLE II

Treated	
No. of roots	Per cent roots stopped growing
28	78
33	39
30	56
72	46
41	63
69	91
273	62†

RESULTS

Table 1 shows that, in every experiment, the percentage of roots which stopped growing was always higher in the group of seedlings with cell division present at the time of radiation. When the averages from all the experiments are compared, the difference is very striking ($P < 0.001$). A study of the lateral roots showed that the seedlings treated at a time after cell division had started were more sensitive to radiation than the seedlings in which there was no cell division at the time of treatment. In the group in which cell division was present, only 60 per cent of the seedlings produced laterals, while in the second group 97 per cent had laterals. In the first group, the initial appearance of the laterals was delayed longer than in the second group. All these facts indicate that the seedlings in the group with cell division suffered the greater amount of radiation injury.

In Table 2, the behavior of the primary roots after treatment with HN2 shows that there was much less difference in sensitivity to this agent between the group with dividing cells and the group with no cell division at the time of exposure to the HN2 solution. The difference is not significant ($0.01 < P < 0.02$). A study of the lateral roots showed that, although the first of these appeared in both groups at the same time, they were more numerous in the group without cell division, showing these seedlings to be slightly more resistant to HN2.

Chart 1 shows the growth curves of the primary roots which died in two typical experiments using Method A (Table 1, Exp. 3, and Table 2, Exp. 3). The shape of the curve made from data from roots treated with HN2 is very similar to the curve made from the data from the x-rayed roots, and the final length of the primary roots is about the same in both cases.

Chart 2 shows the behavior of the primary roots in a typical experiment (Exp. 3) from Table 3. In each experiment of this series, a larger percentage of roots stopped growing in the normal cell division group than in the reduced cell division group. The difference is highly significant ($P < 0.001$). Again, the laterals appeared later in the former group, and at the end of the experiment only 83 per cent of these seedlings had laterals, as compared to the presence of laterals in 99 per cent of the group with reduced mitotic activity. All this evidence indicates that seedlings with a normal mitotic index were more sensitive to radiation than those in which the number of dividing cells was reduced by DNC treatment.

Chart 3 was constructed on the basis of Exp. 3 in the series recorded in Table 4. This table shows

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that when the two groups of seedlings were treated with identical doses of HN2, the group with a normal rate of cell division was only slightly more sensitive than the group with cell division. The use of the two agents, the doses of x-rays and HN2 used in these experiments with 16-hour germinated embryos are biological.

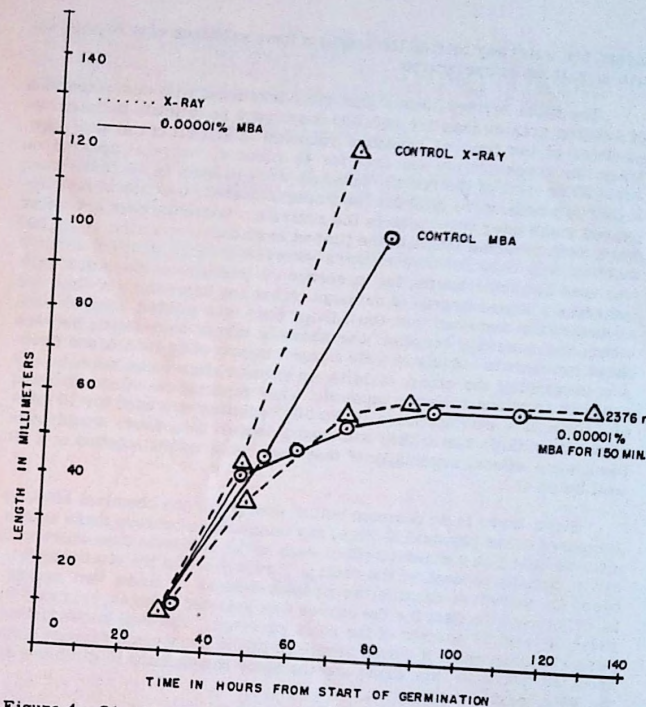


Figure 4. Similarity between the growth of roots following lateral doses of MBA and physical X-rays.

CONCENTRATION OF MBA		TIME OF EXPOSURE	AVERAGE LENGTH OF ROOT AT DEATH	% OF ROOTS SHOWING LATERALS
%	MOLAR			
1×10^{-1}	5.2×10^{-3}	37 MIN.	8	0
1×10^{-2}	5.2×10^{-4}	5 - 45 "	52 ± 1.97 "	0
1×10^{-3}	5.2×10^{-5}	5 "	60 ± 2.15 "	0
		15 - 45 "	51 ± 2.1 "	0
1×10^{-4}	5.2×10^{-6}	45 "	57 ± 1.76 "	50
1×10^{-5}	5.2×10^{-7}	180 "	57 ± 2.09 "	100
1×10^{-6}	5.2×10^{-8}	300 "	NONE DEAD	100

Table 1. Relation between duration of exposure and concentration of MBA solutions.

into two groups, so that one curve could be made by those which survived for four hours and one curve made for those which died. The curve obtained from roots exposed for 45 minutes was found to be lethal to 100 percent of 0.0001 percent MBA. (See Table I.)

of exposing seedlings to a single concentration interval, experiments were set up to show as shown in Table I.

that a solution of 1:1000 inhibited all growth for 37 minutes, but a solution of 1:100,000,000 exposed for five hours. When the 1:100,000 solutions were similar to those obtained with the same concentration, in that exposure early as much damage as exposure for 45 minutes quite certain that our solution was still active at little more damage appeared because the seedlings died in five minutes at this concentration. This made no difference in our results. This study that all of the active MBA present in the solution during the first five minutes. Experi-

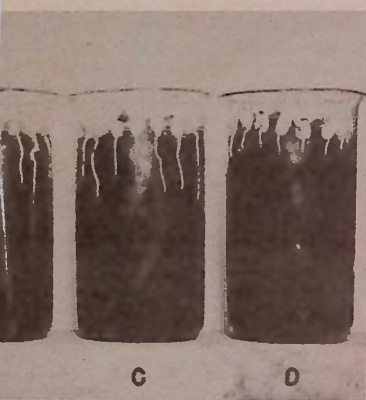


Figure 3. Three days after treatment of seedlings. (a) 0.0001 percent MBA for 25 minutes, showing normal growth in the control beaker (a), while other roots exposed to (b) solution for 45 minutes. This inhibited primary roots and prevented formation of lateral roots. (c) 0.01 percent CuSO_4 for two hours. Compare that these roots are the same length as they are.

- 4 -

Chart 3 was constructed from the experimental data reported in the series recorded in Table 4. This table shows

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ments are underway testing the length of time solutions will remain active at various temperatures.

The table further shows that after treatment with concentrations of solution greater than 1:1,000,000 there were no laterals formed, regardless of the time of exposure required to kill all of the seedlings. When this concentration was used for 45 minutes, laterals appeared on about 50 percent of the roots. Laterals were present in all cases when higher dilutions were used for the times indicated, but they always appeared much later than those in the controls. While the primary roots were about the same length at the time of death either when the 1:100,000 solution was used for five minutes or when the 1:10,000,000 solution was used for three hours, the presence of laterals in the latter case indicates a lesser degree of damage. When the logarithms of time and concentration required for the LD_{50} dose are plotted against each other, the resulting straight line shows a direct correlation between these two factors. Early in 1949 a paper appeared by Novick and Sparrow describing the effect of MBA on mitosis after roots had been exposed to solutions of that compound. They reported no effect on roots of Lillium if a concentration of 5×10^{-2} solution was used for 10 minutes. It is likely that if they had used a longer time there would have been some effect, especially if they had made up the solution at a pH well below 7.

Since there is no common unit of dosage for the chemical MBA as compared to the physical X-rays, any comparison between these agents must be based on a standard effect such as the minimum dose which will kill a certain percent of the roots. Figure 4 shows the similarity between the growth of roots following lateral doses of these two agents. In both cases the data for the curves was selected from experiments in which at least 10 percent of the roots survived. The well known "latent period" during which X-rayed seedlings fail to show much damage lasts about 30 hours in this case, and the same phenomenon is exhibited by the MBA treated roots.

This similarity between the behavior of seedlings following exposure to radiation and MBA solutions is even more striking when one observes the great difference between the behavior of seedlings exposed to minimum lethal doses of MBA in one case and a typical plant poison, such as copper sulphate, in another. After treatment with the copper sulphate (1:10,000 for two hours) there is no further growth of the root, which is in marked contrast to the growth of at least 40 mm after MBA is used. (See Figure 3d).

In summary, the experimental data presented in this paper may warrant the following conclusions:

- (1) In gross appearance the corn roots behave much the same after exposure to lethal doses of X-rays and MBA. In both cases lateral roots are either delayed in their time of appearance or are suppressed entirely, while the primary roots grow to a considerable length before death occurs.
- (2) Solutions of MBA may remain active for a considerable time if prepared from acid distilled water of about pH 5.7. This permits a longer exposure time and thus might cut down errors arising

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from slight variations in the time involved in a given experiment. Furthermore, seedlings may be exposed to high dilutions of the solution for relatively long periods of time, and the results then compared with those obtained by using a high concentration for a short time.

This work is being supported by Contract N8 onr 78300 through the joint sponsorship of the Office of Naval Research and the U. S. Atomic Energy Commission.

The author gratefully acknowledges the assistance of F. J. Michelini.

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that when the two groups of seedlings were treated with identical doses of HN₂, the group with a normal rate of cell division was only slightly more sensitive than the group with cell division reduced by DNC. This difference is not significant, since $0.01 < P < 0.02$. While all the roots had laterals at the end of the experiments, the normal cell division group showed a slightly increased sensitivity to HN₂, as indicated by the laterals in these seedlings appearing somewhat later than in the group with the reduced cell division.

DISCUSSION

A comparison of the "cell division present" columns in Tables 1 and 2 shows that, after the use of these doses of radiation or HN₂, the percentages of roots which stopped growing were nearly the same. The average length of the primary roots at the time of death and the number of seedlings in which lateral root growth was entirely suppressed were approximately equal in both groups. Since about the same amount of injury resulted from

the use of the two agents, the doses of x-rays and HN₂ used in these experiments with 16-hour germinated embryos are biologically equivalent to one another. However, if younger embryos containing no dividing cells were used as the test material, with the same dose of HN₂, a considerably larger dose of x-rays would be required to kill the same percentage of roots.

It will be noted in Table 1 that 3,500 and 4,000 r were used to stop the growth of 73 per cent of the 16-hour germinated embryos, but the results of Table 3 show that only 1,700 and 1,750 r were required to produce the same mortality in the group with the normal rate of mitosis. The greater sensitivity of the older seedlings as shown in Table 3 may be due, in part, to the increase in hydration and general metabolic rate, but it is probable that most of the increased sensitivity can be attributed to the higher mitotic rate.

One obvious reason for the effectiveness of the more dilute solution of HN₂ used to treat the older seedlings (Table 4) is that these roots could ab-

TABLE 1
COMPARISON OF THE EFFECT OF X-RAYS ON CORN EMBRYOS CONTAINING DIVIDING CELLS AND YOUNGER EMBRYOS WITH NO DIVIDING CELLS AT THE TIME OF RADIATION

		CELL DIVISION PRESENT (15 PER CENT)						CELL DIVISION ABSENT					
		Controls		Treated		GERMINATION		Controls		Treated			
EXP. NO.	X-RAY DOSE	GERMINATION TIME (hr.)	No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing	TIME (hr.)	No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing		
1	4,000 r	15	31	10	13	30	11	28	4	15	20		
2	3,500 r	15	—	—	14	43	11	—	—	14	21		
3	4,000 r	16	31	0	29	97	12	31	0	30	20		
4	4,000 r	16	32	0	25	84	12	32	0	29	41		
5	4,000 r	16	32	6	29	86	12	33	0	28	15		
6	4,000 r	16	16	0	32	62	12	16	0	28	10		
Total			142	3.5*	142	73	—	140	0.7*	144	22*		

* Weighted averages.

TABLE 2
COMPARISON OF THE EFFECTS OF HN₂ ON CORN EMBRYOS CONTAINING DIVIDING CELLS AND YOUNGER EMBRYOS WITH NO DIVIDING CELLS AT THE TIME OF TREATMENT

Exp. No.	HN ₂ dose 5.2X 10 ⁻³ M (min.)	GERMINATION TIME (hr.)	CELL DIVISION PRESENT (15 PER CENT)				GERMINATION TIME (hr.)	CELL DIVISION ABSENT				
			Controls		Treated			Controls		Treated		
			No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing		No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing	
1	30	15	29	0	29	45	11	20	10	28	78	
2	30	15	36	0	36	55	11	36	0	33	39	
3	30	16	38	0	33	55	12	35	0	30	56	
4	30	16	33	0	32	62	12	34	0	72	46	
5	30	15	31	0	53	75	11	31	0	41	63	
6*	30	16	18	0	70	97	12	17	0	69	91	
Total			182	0	253	71†	173			1.2†	273	62†

* pH of water higher, HN₂ solution pH 6.6.

† Weighted averages.

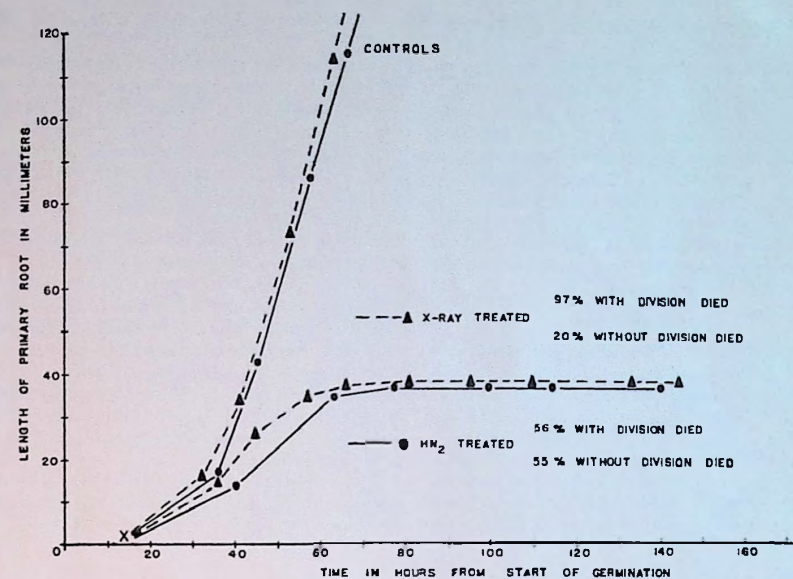


CHART 1.—Growth curves of primary roots of corn seedlings which died after exposure to 4,000 r and 5.2×10^{-4} M HN_2 solution for 30 minutes, respectively.

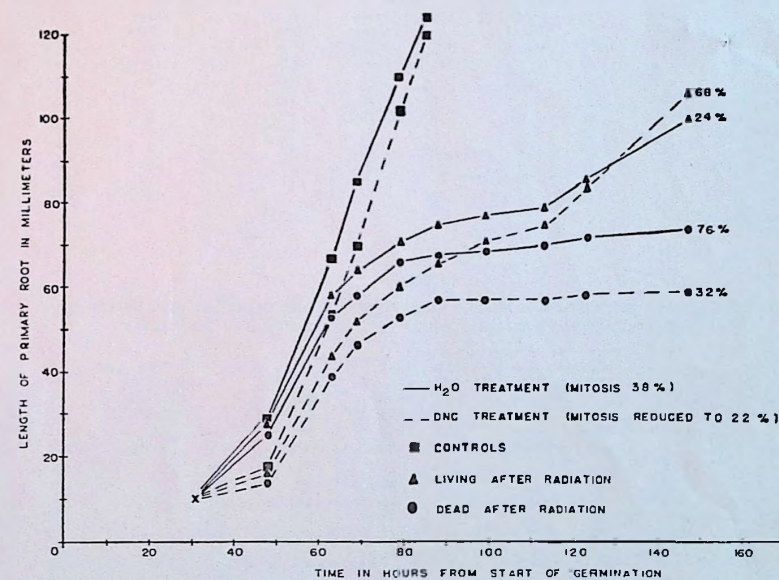


CHART 2.—Growth curves of primary roots of seedlings radiated with 1,700 r after soaking in water (38 per cent mitosis) and after soaking in DNC (22 per cent mitosis).

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sorb the solution freely, while the roots of all the younger embryos (Table 2) were protected by the intact pericarp.

The embryos of the two age groups used in Method A differed from one another not only in the number of dividing cells, but also in that the tissue of the older group was more highly differentiated and had a greater degree of hydration. The last two variables were eliminated in Method B, which made use of seedlings of the same age.

When DNC seedlings were radiated, they were less sensitive than normal seedlings. If the respiration rate had been increased by the DNC pretreatment, one would expect sensitivity to increase also. This tendency may have been present and its effect masked by the effect of decreasing rate of mitosis on lowering sensitivity. The possibility of any interaction between DNC and HN₂ would seem to be ruled out by the results of the following experiment: when HN₂ was dissolved in a 10^{-5} M

TABLE 3
COMPARISON OF THE EFFECT OF X-RAYS ON NORMAL CORN SEEDLINGS WITH 38 PER CENT DIVIDING CELLS AND ON SEEDLINGS TREATED WITH DNC TO REDUCE CELL DIVISION TO 22 PER CENT

Exp. No.	X-ray dose	NORMAL CELL DIVISION (38 PER CENT)		CELL DIVISION REDUCED TO 22 PER CENT BY DNC	
		Controls	Treated	Controls	Treated
		No. of roots	No. of roots	No. of roots	No. of roots
		Per cent roots stopped growing	Per cent roots stopped growing	Per cent roots stopped growing	Per cent roots stopped growing
1	1,750 r	34	39	26	43
2	1,700 r	16	34	20	38
3	1,700 r	18	38	17	37
4	1,700 r	15	30	16	35
Total		83	141	179	153
			72*	1*	31*

* Weighted averages.

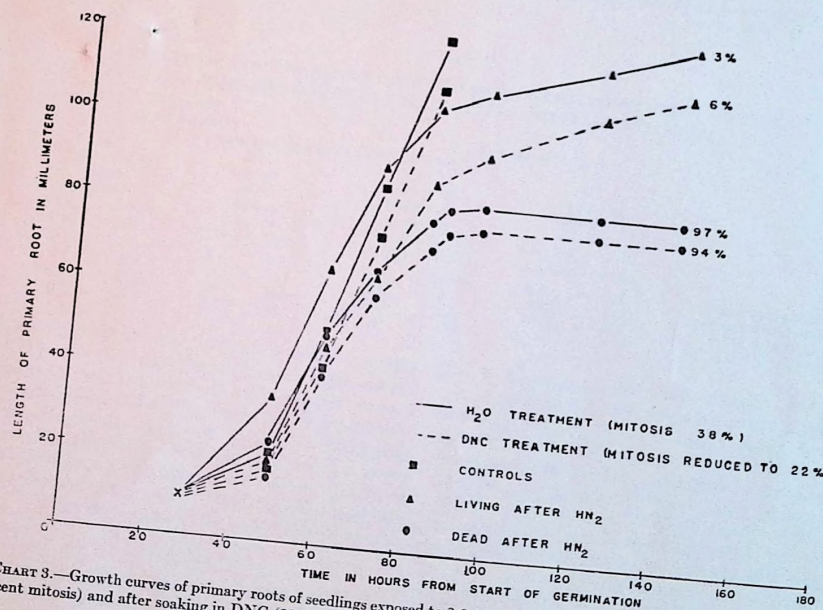


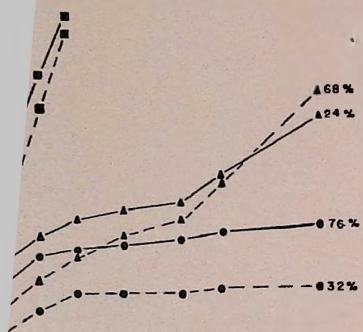
CHART 3.—Growth curves of primary roots of seedlings exposed to 2.6×10^{-5} M HN₂ for 20 minutes after soaking in water (38 per cent mitosis) and after soaking in DNC (22 per cent).

97% WITH DIVISION DIED
20% WITHOUT DIVISION DIED

56% WITH DIVISION DIED
55% WITHOUT DIVISION DIED

HOURS FROM START OF GERMINATION

ings which died after exposure to 4,000 r and 5.2×10^{-5} M HN₂



— H₂O TREATMENT (MITOSIS 38%)
- - - DNC TREATMENT (MITOSIS REDUCED TO 22%)
■ CONTROLS
▲ LIVING AFTER RADIATION
● DEAD AFTER RADIATION

IN HOURS FROM START OF GERMINATION

Seedlings radiated with 1,700 r after soaking in water (38 per cent mitosis)

solution of DNC instead of water, seedlings exposed to this solution behaved the same as seedlings treated with the same concentration of HN₂ made up in water.

Table 5 summarizes the data presented in the other tables. The mortality figures in line 2 show that, with the use of either method, corn root tissue with the greater amount of cell division present at the time of radiation was much more sensitive to x-rays than the same tissue with less cell division. In line 3 the figures from both methods

of Henshaw and Francis (4), who reported similar results with wheat seedlings. The fact that the roots whose rate of cell division was reduced by DNC were less sensitive to x-rays than normal roots of the same age is also in agreement with the general conception that sensitivity to radiation is usually directly proportional to the mitotic index of the tissue.

The experimental data presented here confirm the statement by Strong (7) that nitrogen mustard seemed to affect cells even before there was any

TABLE 4
COMPARISON OF THE EFFECT OF HN₂ ON NORMAL CORN SEEDLINGS AND ON SEEDLINGS TREATED WITH DNC TO REDUCE CELL DIVISION

Exp. no.	HN ₂ dose 2.6×10^{-6} M (min.)	NORMAL CELL DIVISION (38 PER CENT)		CELL DIVISION REDUCED TO 22 PER CENT BY DNC			
		Controls		Treated		Controls	
		No. of roots	Per cent stopped growing	No. of roots	Per cent stopped growing	No. of roots	Per cent stopped growing
1	16	17	0	46	17	17	0
2	18	18	0	30	63	17	0
3*	20	19	0	69	97	18	0
4	20	38	6	54	92	34	0
Total		88	2†	229	53†	86	0

* pH of water higher, HN₂ solution pH 6.6.

† Weighted averages.

TABLE 5
COMPARISON OF THE EFFECTS OF X-RAYS OR HN₂ WHEN APPLIED TO TISSUE WITH VARYING AMOUNTS OF CELL DIVISION OBTAINED BY TWO DIFFERENT METHODS

	METHOD A (TABLES 1 AND 2)				METHOD B (TABLES 3 AND 4)			
	15- or 16-hr. germinated embryos		11- or 12-hr. germinated embryos		PER CENT MITOSIS AT TIME OF TREATMENT		22 DNC-treated 48-hr. seedlings	
	No. of plants	Per cent mortality	No. of plants	Per cent mortality	38 Normal 48-hr. seedlings	Per cent mortality	No. of plants	Per cent mortality
Controls for radiation	142	3.5	140	0.7	83	0	170	1
Treated with X-rays	142	73.0	144	22.0	141	72	153	31
Treated with HN ₂	253	71.0	273	62.0	229	53	243	46
Controls for HN ₂	182	0	173	1.2	88	2	80	0

show that, when seedlings were exposed to HN₂, the variation in the amount of cell division at the time of exposure made no significant difference in the sensitivity of the roots. These results show that under the conditions of these experiments differences in mitotic activity during exposure affected sensitivity to HN₂ to a negligible degree, while the radiosensitivity of the same tissue increased with the amount of cell division present at the time of exposure to x-rays.

The data indicating a marked rise in sensitivity of embryo roots to radiation, at about the time that mitosis commences, agree with the findings

evidence of cell division. It may be that, although the seedlings showed the effects of x-rays and HN₂ in many similar ways, the action of the two agents was different when the reaction of resting cells was compared to that of dividing cells. Further experimentation may show whether this difference is mainly in the initial damage or whether the dividing cells are better able to recover after exposure to HN₂ than after x-ray treatment.

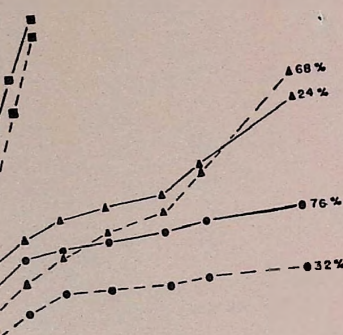
SUMMARY

1. Two methods were used to obtain corn seedlings with little or no cell division for comparison

97% WITH DIVISION DIED
20% WITHOUT DIVISION DIED

56% WITH DIVISION DIED
55% WITHOUT DIVISION DIED

SEEDLINGS WHICH DIED AFTER EXPOSURE TO 4,000 r AND 5.2×10^{-6} M HN₂



— H₂O TREATMENT (MITOSIS 38%)
--- DNC TREATMENT (MITOSIS REDUCED TO 22%)
■ CONTROLS
▲ LIVING AFTER RADIATION
● DEAD AFTER RADIATION

SEEDLINGS RADIATED WITH 1,700 r AFTER SOAKING IN WATER (38 PER CENT MITOSIS)

ings ex- of Henshaw and Francis (4), who reported similar
is seed- results with wheat seedlings. The fact that the
of HN₂ roots whose rate of cell division was reduced by
DNC were less sensitive to x-rays than normal
roots of the same age is also in agreement with the
general conception that sensitivity to radiation is
usually directly proportional to the mitotic index
of the tissue.

The experimental data presented here confirm
the statement by Strong (7) that nitrogen mustard
seemed to affect cells even before there was any

TABLE 4

HN₂ ON NORMAL CORN SEEDLINGS AND ON SEEDLINGS
TREATED WITH DNC TO REDUCE CELL DIVISION

DIVISION (38 PER CENT)	CELL DIVISION REDUCED TO 22 PER CENT BY DNC			
	Treated	Controls	Treated	
	No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing
1	46	17	17	0
2	50	65	17	0
3	69	97	18	0
4	84	32	34	0
5	229	53†	86	0
6			243	46†

TABLE 5

X-RAYS OR HN₂ WHEN APPLIED TO TISSUE WITH VARYING
EFFECTS OBTAINED BY TWO DIFFERENT METHODS

METHOD A (TABLES 1 AND 2)				METHOD B (TABLES 3 AND 4)			
PER CENT MITOSIS AT TIME OF TREATMENT				PER CENT MITOSIS AT TIME OF TREATMENT			
0				38			
11- or 12-hr. germinated embryos				Normal 48-hr. seedlings			
22				DNC-treated 48-hr. seedlings			
Plant	No. of plants	Per cent mortality		No. of plants	Per cent mortality	No. of plants	Per cent mortality
5	140	0.7		88	0	179	1
0	144	22.0		141	72	153	31
0	273	62.0		229	53	243	46
	173	1.2		88	2	86	0

1 to HN₂, evidence of cell division. It may be that, although
the seedlings showed the effects of x-rays and HN₂
in many similar ways, the action of the two agents
was different when the reaction of resting cells was
compared to that of dividing cells. Further ex-
perimentation may show whether this difference
is mainly in the initial damage or whether the
dividing cells are better able to recover after ex-
posure to HN₂ than after x-ray treatment.

SUMMARY

sensitivity 1. Two methods were used to obtain corn seed-
t the time lings with little or no cell division for comparison
e findings

RUSSELL AND MICHELINI—Relation of Mitosis to Effects of X-Rays and a Mustard 693

with similar tissue containing a higher percentage
of dividing cells:

a) Embryos were germinated 12 hours for root
tips containing no cell division and 16 hours for
tips with about 15 per cent of the meristematic
cells dividing.

b) Older seedlings were treated with 3,5-dini-
tro-o-cresol, which reduced the number of dividing
cells in the root tip from the normal 38 per cent to
22 per cent.

2. When either method was used, the group of
seedlings with the higher mitotic index was only
slightly more sensitive to the effects of methyl-
bis(β-chloroethyl)amine hydrochloride (HN₂)
than the group with the lower index.

3. When either method was used, the group of
seedlings which had the greater number of divid-
ing cells at the time of exposure to x-rays was dam-
aged significantly more than the group where there
were fewer dividing cells.

4. Under the same experimental conditions,
corn root tissue showed a significant increase in
sensitivity to x-radiation with increase in the num-
ber of dividing cells, whereas no such relationship
existed with the action of HN₂.

5. The ratios between biologically equivalent
doses of x-rays and a mustard compound may vary
widely for the same tissue if its mitotic index is
raised or lowered.

ACKNOWLEDGMENTS

We wish to acknowledge the courtesy of the Delaware Hos-
pital which furnished the radiation.

REFERENCES

1. CLOWES, G. H. A., and KRAHL, M. E. Action of Dinitro Compounds on Sea Urchin Eggs. *Science*, 80:384-85, 1934.
2. FRIEDENWALD, J. S., and BUSCHKE, W. Studies on the Physiology, Biochemistry, and Cytopathology of the Cornea in Relation to Injury by Mustard Gas and Allied Toxic Agents. V. Nuclear Fragmentation Produced by Mustard and Nitrogen Mustards in the Corneal Epithelium. *Bull. Johns Hopkins Hospital*, 82:161-78, 1948.
3. GILMAN, A., and PHILLIPS, F. S. The Biological Actions and Therapeutic Applications of the β-Chloroethyl Amines and Sulfides. *Science*, 103:400-15, 1946.
4. HENSHAW, P. S., and FRANCIS, D. S. A Consideration of the Biological Factors Influencing the Radiosensitivity of Cells. *J. Cell. & Comp. Physiol.*, 7:173-95, 1935.
5. RUSSELL, M. A. Effects of X-Rays on Zea Mays. *Plant Physiol.*, 12:117-33, 1937.
6. Nitrogen Mustard and X-Ray Research, pp. 1-7. Research Reviews (Office of Naval Research), May, 1950.
7. STRONG, L. C. Induction of Mutations by a Carcinogen. pp. 486-99. *Proc. 8th Internat. Cong. of Gen.*, 1948.

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VISION (38 PER CENT)		CELL DIVISION REDUCED TO 22 PER CENT BY DNC			
Treated		Controls		Treated	
Per cent roots stopped growing		Per cent roots stopped growing		Per cent roots stopped growing	
No. of roots		No. of roots		No. of roots	
46	17	17	0	57	8
30	63	17	0	30	46
69	97	18	0	71	94
84	32	34	0	85	20
229	53†	86	0	243	46†

TABLE 5
X-RAYS OR HN2 WHEN APPLIED TO TISSUE WITH VARYING
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sensitivity
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ae findings

SUMMARY

1. Two methods were used to obtain corn seed-
lings with little or no cell division for comparison

► inhibitors, made by Celgene and by Merck, another New Jersey-based firm, are being used to treat a rare illness called cutaneous T-cell lymphoma.

More recently, researchers led by Stephen Baylin at Johns Hopkins School of Medicine, in Baltimore, have shown that a combination of a histone-deacetylase inhibitor and azacitidine slowed tumour growth in some people with advanced lung cancer. This result was notable for two reasons. It was the first time epigenetic drugs had been deployed successfully against a solid tumour, rather than a leukaemia or a lymphoma (solid tumours are harder to treat, because the drug has to penetrate them). And, second, some of the participants in Dr Baylin's study who did not show much response to the trial itself then went on to show an unexpectedly good reaction to the routine chemotherapeutic drugs which were employed on them next. Although it is too early to say for sure, Dr Baylin speculates that his epigenetic drugs altered the tumour cells in some lasting way that made them more susceptible to standard chemotherapy.

That is quite possible. Unlike other forms of gene regulation (those involving transcription factors, for example), epigenetic changes are passed on during cell division to daughter and granddaughter cells until they are actively erased. Once erased, though, they do not return. It might therefore be that epigenetic therapies can effect changes which stop a cancer growing without having to kill all its cells.

That, indeed, appears to be what is happening in the case of GSX2816126. If it is, it would truly be a conceptual breakthrough, and epigenetics might justly take its place alongside genetics in the analysis and treatment of cancer. ■

Animal testing

Be nice to mice...

...and they may return the favour

ONLY one drug of every ten successfully tested in laboratory animals ends up working in people. One reason, of course, is that mice are not men. Another, though, might have to do with the fact that whereas human patients are afforded all manner of creature comforts, their animal proxies are not.

Although medical science's favourite critters relish temperatures of a little over 30°C, laboratories routinely keep them at five or ten degrees below that. This is not in order to torture the beasts but, rather, because when kept warm they are unman-



Home, sweet home

ageably aggressive. The downside is that they have to eat more than they otherwise would, in order to keep their bodies warm. That changes their physiology. And that in turn alters the way they metabolise drugs, with possibly confusing results.

Joseph Garner, of Stanford University, thinks the answer is to keep the labs cool, but let mice cope with the low temperatures as they do in their natural habitat: not by eating more but by building nests. So far, though, no one has a clear idea of how much nesting material is needed to keep mice happy. Dr Garner and his colleagues therefore decided to find out. They have just reported their results in the *Public Library of Science*.

Dr Garner and his team let each of their mice, 36 males and as many females from three strains commonly used in trials, roam free in two cages connected by a narrow tube. One cage was kept constant at one of six temperatures between 20°C and 35°C. The other was maintained at 20°C but was stocked with up to ten grams of finely shredded paper, which the mice could use to weave a nest. The idea was to check whether the animals would rather build a nest in the cooler cage or move to the warmer one, possibly tugging nesting material along with them strand by strand.

The researchers found that the rodents' preferences varied slightly between strains, as well as between sexes (with females partial to higher temperatures, possibly because of their thinner protective layer of fat), confirming that there is no single set of conditions in which all mice feel cosy. In general, though, with little nesting material around, the animals laboriously carried strands of paper over to the warmer spot, one or two at a time. But leave at least six grams of paper in the chilly cage, and many mice will prefer instead to brave the cold and build a nest there. That seems a small price to pay for better drug trials. ■

Cosmic-ray tomography

Musing on muons

How to detect smuggled uranium and plutonium using muons

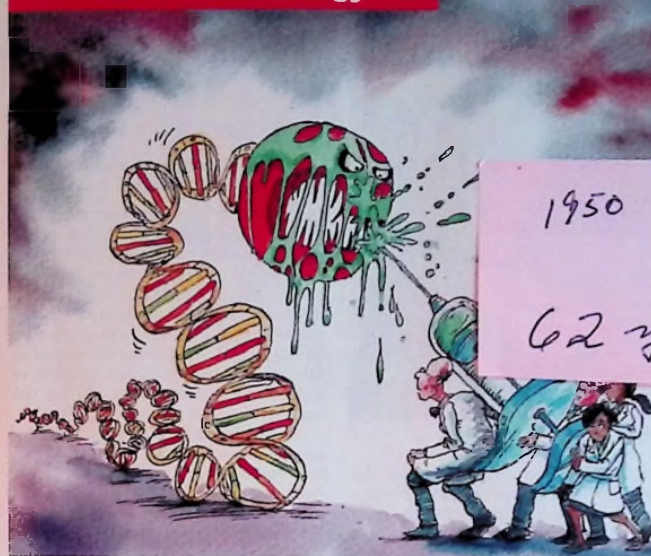
AMERICANS may no longer fret about being showered with nuclear-tipped ballistic missiles, but the idea that an atom bomb might enter their country in a shipping container or on the back of a lorry is still one that keeps the security services awake at night. They may sleep more easily, though, if an idea being developed by Michael Staib of the Florida Institute of Technology, in Melbourne, and his colleagues comes to fruition.

Dr Staib is using naturally generated subatomic particles called muons to look inside places where such bombs, or the nuclear explosives needed to make them, may have been hidden by smugglers. At first sight this seems crazy. The muons in question drizzle down from the atmosphere at the rate of only one per square centimetre a minute. But, as Mr Staib told a meeting in Atlanta of the American Physical Society on April 2nd, this is enough for a practical muon scanner.

Muons are like electrons, though heavier and unstable. They are produced when cosmic rays (fast-moving atomic nuclei from space) hit the atmosphere. The reason they might be useful for detecting nuclear explosives is that they are scattered more by heavy atomic nuclei, such as those of uranium and plutonium, than by lighter ones—even including relatively heavy elements such as lead. Clever electronics can tell the difference. Someone wanting to smuggle uranium or plutonium might shield their contraband from detection by a Geiger counter using lead. But that would be no shield against detection by muons.

The original idea, dreamed up in 2003 at the Los Alamos National Laboratory in New Mexico, was to use detectors called drift tubes to track muons through a cargo. Decision Sciences, a company spun out of that effort in 2005, has been refining this approach since then. It says it will install a container-size demonstration unit in a port in the Bahamas this summer, though it will not reveal any technical details.

Drift tubes, however, are unwieldy. They may also be unable to pick out smaller lumps of contraband. Mr Staib and his colleagues think they can do better. They have been working with another type of detector, called a gas electron multiplier (GEM). GEMs are a tenth of the size of drift tubes, and consist of perforated foil embedded in a thin plate filled with gas. As a muon passes through, it dislodges electrons from the gas, leaving a trail which the ►



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Cancer and epigenetics

Cancer's epicentre

CHICAGO

New understanding of how cancers work is yielding new treatments

THE biggest conceptual breakthrough in the war on cancer was the realisation by the 1980s that it is always a genetic disease. Sometimes the genetic flaw is inherited. Sometimes it is the result of exposure to an outside agent such as tobacco smoke or radioactivity. Sometimes it is plain bad luck; a miscopying of a piece of DNA during the normal process of cell division.

Turning that breakthrough into medicine, though, is hard. No one has worked out how to repair DNA directly. It is, rather, a question of discovering the biochemical consequences of the genetic damage and trying to deal with those instead. But recently, another pattern has emerged. It is too early to call it a breakthrough as significant as the cancer-is-caused-by-broken-genes finding, but it might be.

The pattern in question is that many of the genes whose breakage leads to cancer are themselves involved in a specific sort of genetic regulation, known as epigenetics. This switches genes on and off by plastering either their DNA or the proteins which support that DNA in chromosomes with clusters of atoms called methyl and acetyl groups. The nature of these reactions means epigenetic processes are susceptible to chemical intervention in a way

that genetic mutations are not. They are, in other words, open to drug treatment. And that is why epigenetics was the subject of a particularly interesting session, held on April 1st, at a meeting of the American Association for Cancer Research in Chicago.

A problem of overregulation

Dash Dhanak, who leads the epigenetics research group at GlaxoSmithKline, one of the world's biggest drug companies, described to the meeting his efforts to develop a substance that will inhibit the activity of an enzyme called EZH2. This enzyme attaches methyl groups to histone proteins, which are part of the chromosomal packaging. A lot of lymphomas—cancers of the immune system—are caused by mutations that make EZH2 overactive. Such overactivity methylates histones more than they should be and thus silences the genes they surround, including so-called tumour-suppressor genes whose job is to stop the uncontrolled cell growth that causes cancer.

When Dr Dhanak and his colleagues treated lymphoma cells with a newly developed inhibitor, currently referred to by the unmemorable name GSK2816126, they found that the amount of histone overmethylation declined dramatically. And

when they treated both cell cultures and laboratory animals with GSK2816126, they found it also reduces the proliferation of tumour cells while, crucially, having no apparent effect on nearby normal cells.

James Bradner of the Dana-Farber Cancer Institute, in Boston, described a second epigenetic approach to treating cancer. His group have shown that a substance known as JQ1, which inhibits an epigenetic regulator called BRD4, blocks the activity of a gene by the name of *Myc*. *Myc* encodes a protein called a transcription factor that is another part of the DNA-regulation system. This particular transcription factor is involved in the expression of about 15% of human genes. Not surprisingly, then, when it goes wrong it is one of the most common causes of cancer.

Despite numerous attempts, researchers have been unable to find a way to block the activity of *Myc* directly. Dr Bradner, however, reasoned that blocking BRD4, which is one of *Myc*'s collaborators, might do the job indirectly. To test this thought he and his colleagues treated mice suffering from *Myc*-driven myeloma with JQ1. And it worked. JQ1, they found, shut down *Myc*-activated genes and slowed the proliferation of myeloma cells.

Although neither GSK2816126 nor JQ1 is ready for human trials, two other sorts of epigenetic drugs are already on the market. DNA-demethylating agents, in the form of azacitidine, sold as Vidaza by Celgene, of Summit, New Jersey, and decitabine, sold as Dacogen by Eisai, a Japanese company, are used to treat myelodysplastic syndromes, the precursors of acute myelogenous leukaemia. And histone-deacetylase

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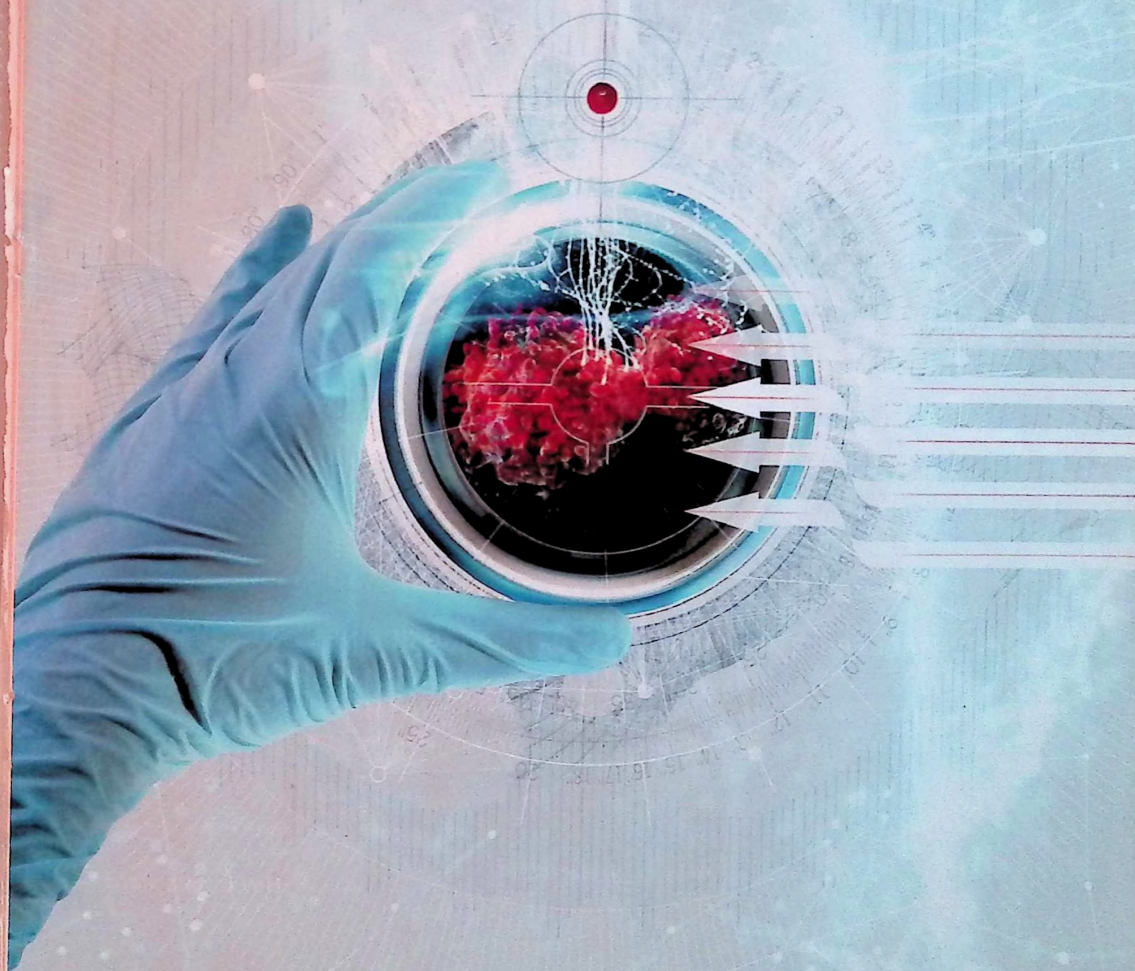
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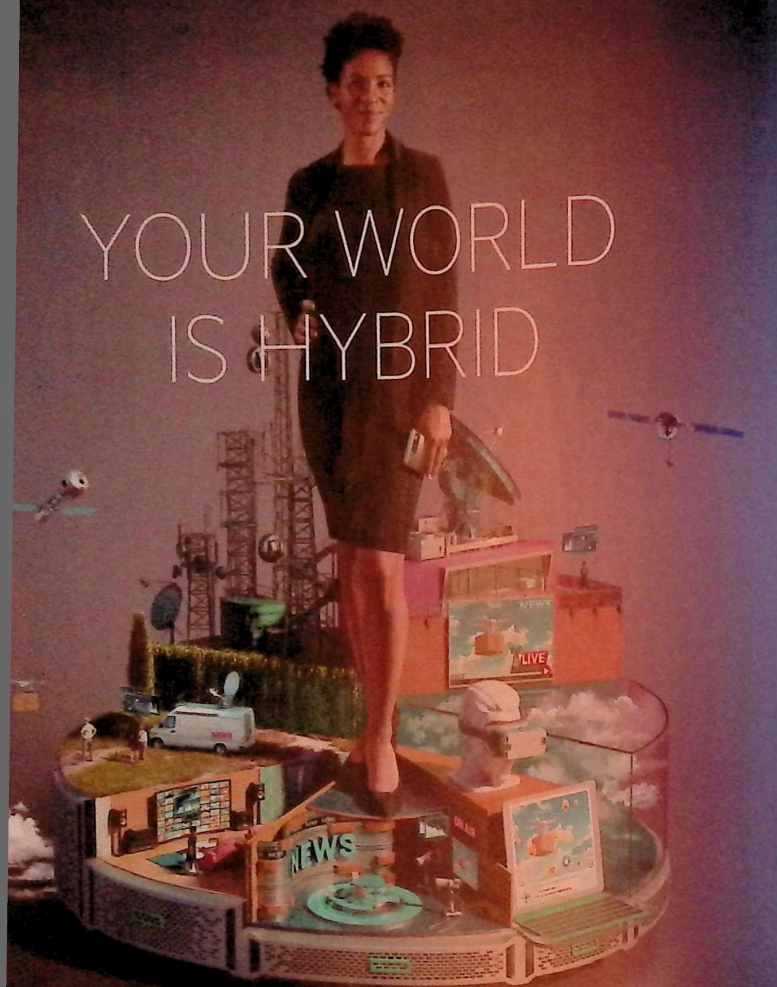
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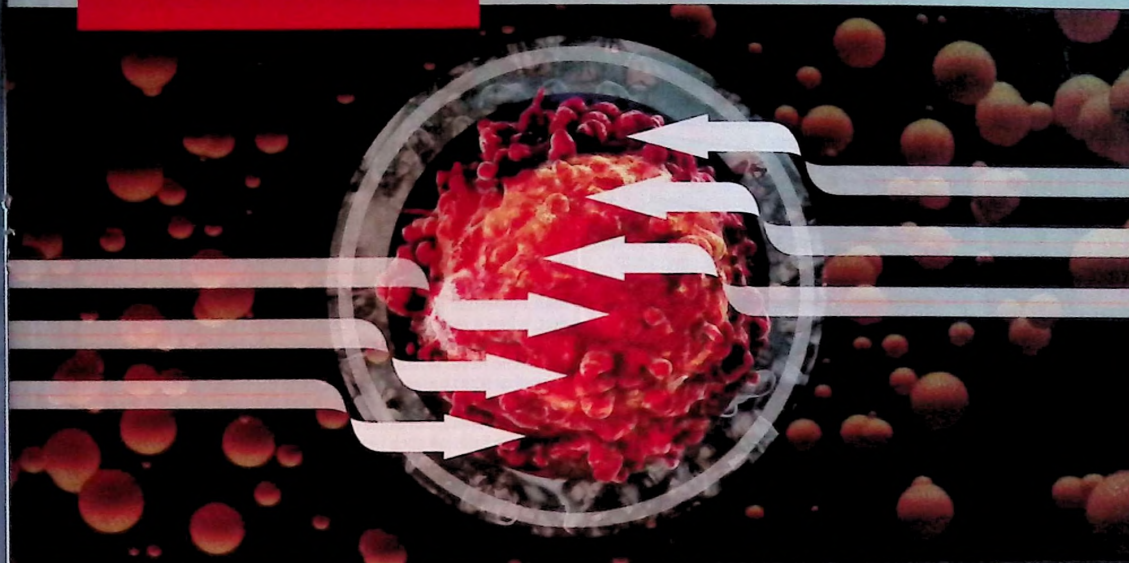
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Treating cancer



A new era of cancer treatment

Targeting tumours

In rich countries half of cancers are now survivable. And better understanding means that more cures are coming, says Natasha Loder

HERE are few whose lives have not been touched by cancer. It cuts down friends, loved ones, siblings, spouses, parents and children. And it does so more than it used to. A generation ago, one in three people in the rich world could expect one day to hear the fateful words, "I'm afraid you have cancer." In some countries it is now approaching one in two. The longer other things do not kill you, the more of the wear and tear that leads to cancer your cells accumulate. Live long enough and it will be the reward.

Worldwide, cancer is the second leading cause of

death after heart disease; it killed 8.8m people in 2015, three-quarters of them in low- and middle-income countries. Between 2005 and 2015 the number of cases increased by 33%, mostly owing to the combined effects of ageing and population growth. New cases are expected to increase by 70% in the next 20 years.

Set against this rise is the fact that, in rich countries, cancer is becoming more survivable. Today 67% of patients in America will survive for at least five years. Different cancers fare differently, as do different sorts of patients—cancer has proved more treatable in children than in adults. Some cancers, such as that of the pancreas, have seen barely any improvement. But there are general grounds for optimism.

New research tools, such as easily generated antibodies, rapid gene sequencing and ever easier genetic engineering, have revolutionised biologists' understanding of cancer. This understanding has allowed more specific approaches to the disease to be developed, and the trend will continue. What is more, the tools of molecular biology have moved out of the lab and into the clinic. Genetic tests are used to find the precise vulnerabilities of a particular patient's cancer. Antibodies attack the specific molecules that have gone haywire. The cells of patients with cancer are engineered to better fight the disease.

And in the current decade a whole new branch of therapy has sprung up. Unshackling the immune system's response to cancer, once a pipe dream, has be-

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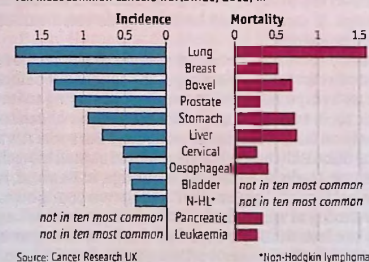
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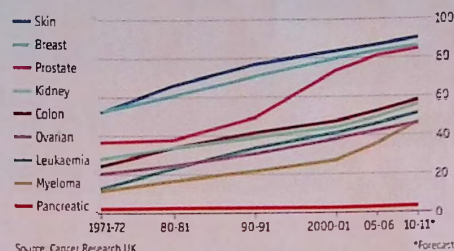
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TECHNOLOGY QUARTERLY | Treating cancer

Living longer

England and Wales, five-year relative survival rate by type of cancer, %



ome practical medicine, with approved therapies for eight kinds of cancer. The excitement at oncology conferences is palpable.

As these advances have arrived regulators have increased the speed with which treatments for life-threatening diseases are approved. This is in some ways a mixed blessing—some expensive drugs with little if any benefit are nevertheless getting to market. But it has encouraged an unprecedented wave of investment and innovation. The pipeline of oncology drugs in clinical development has grown 45% in the past decade. There are currently about 600 drugs under development at biotech and pharma companies.

The picture is not uniformly rosy. In both treatment and prevention the poor are ill served. Basic chemotherapy and pain relief is difficult to come by in many parts of the world. The failures are not limited to poor countries. Cancers due to bad diet, obesity, alcohol abuse and smoking could all be reduced a great deal in wealthy ones. And while vaccination against human papilloma virus is routine in Rwanda, it is still limited in America—which means thousands of American women will face cervical cancers they could have avoided in years to come.

But if some low-hanging fruit still go tragically, lethally unpicked, progress is not merely possible. It is happening. ■

Unruly origins

Beginnings, and how to spot them

It all starts with a single cell

DIANE MILLEY, a teacher, remembers getting the small, dry cough just before school broke up for the summer in 2013. She wasn't worried: she considered herself generally healthy—she ran three times a week and went to the gym. Her doctor in Bradford, Massachusetts, put her on a course of antibiotics. When they didn't work she had an x-ray. It showed nodules across her lungs. A bronchoscopy was ordered to retrieve a tissue sample from her lung. As she came round from the anaesthetic she remembers overhearing two medical staff talking. One said "It's malignant." She had late-stage lung cancer.

Ms Milley's body, like all human bodies, contained tens of trillions of copies of her genome. In theory, all those copies should be more or less the same. In practice, over the years, they all get knocked around in different ways. The oxygen that powers cell metabolism damages the DNA on which the genes are stored as a

matter of course; so do background radiation and exposure to the many low-level carcinogens; so do sunlight and infection with viruses; so do choices about diet and recreational drugs, notably alcohol and tobacco (from which Ms Milley abstained).

The vast bulk of this damage is quickly fixed by DNA-repair enzymes; fewer than one mutation in a thousand persists. But wear and tear builds up. Many such changes make little or no difference. A few will be of consequence to the cell concerned, reducing or eliminating its capacity to do its job. But the loss of a single cell's contribution matters not a jot.

There are some genes, though, where uncorrected damage can matter a lot. Foremost are the genes which control cell growth, such as *HER2*, which tells the cell how to make a protein called human epidermal-growth-factor receptor type 2. This is a protein that, when it sees a particular hormone, tells the cell it is in to divide. Mutations in the *HER2* gene can make cells proliferate when there is no need. When they do so to their daughter cells, which will share that *HER2* mutation, will go on to do the same.

Among some 20,000 genes in the genome there are dozens which, like *HER2*, can cause unwanted cell division when they go wrong. To forestall such problems there are various tumour-suppressor genes whose job is to make sure that cells damaged in this way shut themselves down. The best known is the gene for a protein called p53, which stops cells from reproducing if their DNA is damaged. But these tumour-suppressor genes, too, are subject to mutation.

The numbers game

Thus over time, as genetic damage accumulates, the likelihood rises that somewhere in the body's trillions of cells there is one that has, through five or six mutations in key genes, developed the ability to grow without check. This likelihood is not the same for everyone. Some people start off with quirks in their genome which make them more susceptible. Take the genes *BRCA1* and *BRCA2*, which describe proteins that repair DNA; people who inherit a damaged version of one or the other face a higher risk of cancer (in particular, breast and ovarian cancer) because, with one crucial function already compromised, it takes fewer mutations for a tumour to get going.

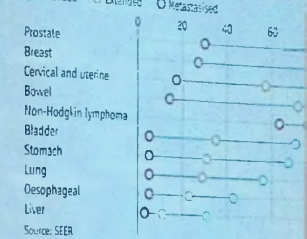
Once a cancer has begun its unruly growth it will pick up more and more mutations: the cancer genome project at the Sanger Institute, outside Cambridge in England, has found that cancers can have as few as ten mutations or as many as a few hundred. Though all the cells in the cancer are descended from one parent cell, they become increasingly diverse over time. Some cells come loose and start new tumours of their own elsewhere. The body's immune system will often recognise that something is amiss and try to fight the cancer and slow its spread. Sometimes it wins, stopping the cancer or killing it. Sometimes it doesn't.

When Ms Milley's cancer was diagnosed all the things that could go wrong already had; the tumour was well developed and had spread through the lung and beyond. It would have been far better for her if it had been diagnosed earlier (see chart on next page). But with lung cancer, as with many other forms of the disease, there are often few symptoms until the disease is already at an advanced stage. If cancer could be reliably detected earlier, many lives might be saved.

In some wealthy countries, some cancers—for example, those of the breast, prostate and cervix—are regularly sought out before they start to cause symptoms. Now researchers are trying to improve diagnostic tools even further, so that more types of cancer can be found early on (and with greater reliability). For some it is a terribly personal hunt. Billy Boyle, the president of a small biotech company, Owlsone Medical, based in Cambridge, in England, is one of them. He lost his wife Kate, mother to their two young boys, on Christmas morning in 2014. She died of colon cancer that had been picked up too late. Mr Boyle says that if colorectal cancer is detected early, 95% of sufferers survive. Only 6% survive if the can-

First come, first saved

United States, five-year relative survival rate by state at diagnosis



Mr Boyle wants to detect cancer on the breath using a mass spectrometer—a gadget that weighs chemical compounds by ionizing them and measuring their mass-to-charge ratio. The breath contains a wide range of organic molecules that reflect what's going on in the body's metabolism. Cancers, which affect the metabolic pathways, change the pattern of molecules in the breath. "Owlstone's system is very small—it fits on a coin—it is sensitive, identifying molecules at a level per billion. The firm hopes that when it has identified 'fingerprints' associated with particular cancers it can detect the disease earlier than other tests do."

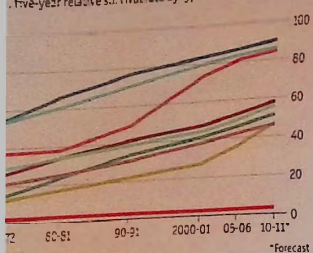
Improved diagnostics can do more than pinpoint. They can also reveal the cancers' weak points. Cancer drugs work in different ways, some will do tumour with one set of mutations but one that has others by some other pathway unsathed. Troy Cox, head of Molecular Diagnostics at Genzyme, a biotechnology company based in Cambridge, Massachusetts, says that in America 14 cancer drug companies are developing "companion diagnostics"—tests that show whether a patient is likely to be susceptible to them or not. Ms Milley's cancer, for example, turned out to harbour a mutation which could be treated with a drug that targets that specific protein (see next section).

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David Hyman, at the Memorial Sloan Kettering in New York, worked on a trial of an experimental nib, that was expected to work in cancers where NTRK1 had undergone a specific mutation. Because it is found in less than 1% of all cancer patients, recruiting for the trial was a "Herculean effort", he says. It was a tough sell. The drug was tested on 50 patients with 17 different tumour types responded to the drug.

Aside from picking the right drug to reveal more about the outcome and risks of any cancer—something that is useful for deciding whether

Five-year relative survival rate by type of cancer, %



medicine, with approved therapies for eight kinds of cancer. The most common cancer, breast cancer, has arrived at a point where the treatments for life-threatening diseases are as good as a mixed blessing—some expensive, some benefit are nevertheless getting to market. The most common cancer, breast cancer, has arrived at a point where the treatments for life-threatening diseases are as good as a mixed blessing—some expensive, some benefit are nevertheless getting to market. The most common cancer, breast cancer, has arrived at a point where the treatments for life-threatening diseases are as good as a mixed blessing—some expensive, some benefit are nevertheless getting to market.

Diagnoses, and how to treat them

From a single cell

MILLEY, a teacher, remembers getting the small, lump just before school broke up for the summer in 1978. She wasn't worried: she considered herself generally healthy—she ran three times a week and went to the gym. Her doctor in Bradford, Massachusetts, put her on a course of antibiotics. When they didn't work she had a red nodule across her lungs. A bronchoscopy was done and a tissue sample from her lung. As she came anaesthetic she remembers overhearing two medical students. One said "It's malignant." She had late-stage lung

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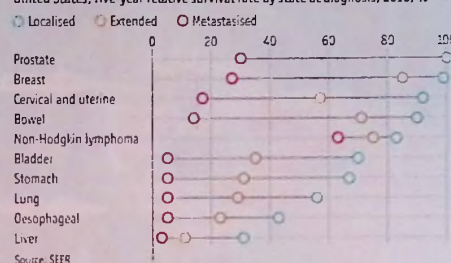
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First come, first saved

United States, five-year relative survival rate by state at diagnosis, 2016, %



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Improved diagnostics can do more than pick up cancers sooner. They can also reveal the cancers' weaknesses. Because cancer drugs work in different ways, some will do well against a tumour with one set of mutations but one that has become cancerous by some other pathway unscathed. Troy Cox, head of Foundation Medicine, a diagnostics company based in Cambridge, Massachusetts, says that in America 14 cancer drugs now have "companion diagnostics"—tests that show whether a cancer is likely to be susceptible to them or not. Ms Milley's lung cancer, for example, turned out to harbour a mutation which meant she could be treated with a drug that targets that specific protein (see next section).

So far, such genetic tests are used when planning therapy for 50% to 60% of solid tumours, according to Foundation. New drugs, new understanding of cancer mechanisms and new technologies that can scan many genes for mutations at once mean such testing will be more informative in the near future. Many, including England's chief medical officer, Sally Davies, want cancer patients to be routinely offered genetic screening of their tumours. Foundation and ThermoFisher, a diagnostics firm in Waltham, Massachusetts, are hoping to encourage this by offering every gene of interest on a mass-produced chip. Some of these mutations will help doctors pick the best drugs for that particular cancer, others may indicate how it is likely to develop. The tests would also identify mutations for which there is not yet an approved therapy—but for which there is one in clinical trials.

David Hyman, at the Memorial Sloan Kettering Cancer Centre in New York, worked on a trial of an experimental drug, larotrectinib, that was expected to work in cancers where a gene called *NTRK1* had undergone a specific mutation. Because that mutation is found in less than 1% of all cancer patients, recruiting people to the trial was a "Herculean effort," he says. It was worth it, though. The drug was tested on 50 patients with 17 different types of tumour. In results published in June, 78% of patients with 12 different tumour types responded to the drug.

Aside from picking the right drugs, genetic tests are also starting to reveal more about the outcome and risks of any individual cancer—something that is useful for deciding whether a cancer needs

to be treated at all. The MammaPrint test, made by Agendia, based in Amsterdam, analyses the activity of genes in early-stage breast cancer. If women with early-stage breast cancer were routinely tested in this way, those who will not need chemotherapy after surgery could be picked out (a recent study of patients found 58% to be in this category). A similar test is available for prostate cancer from the firm Myriad Genetics, based in Salt Lake City, Utah. A recent study suggests that people who have inherited a mutation in the *p53* tumour-suppressor gene might be well advised to have whole-body MRI scans to screen for cancers, since their unsafe-guarded cells are at particular risk.

Identifying genes from tumours normally means retrieving cancer cells via biopsies. This is invasive and often done only once in the course of the disease. But cancers are both heterogeneous and labile; elsewhere in a tumour, and later in a tumour's progression, things may look different.

These challenges are now being tackled with blood tests, a technique termed "liquid biopsy". Tumours shed DNA into the blood, and these circulating fragments of DNA can be tested for mutations. Regularly testing this DNA could be a way of keeping track of a tumour's mutations. The Institute for Cancer Research, based in London, recently showed that it could use a liquid biopsy to pick out whether a patient was likely to benefit from a new type of drug called a PARP inhibitor. Using liquid biopsies the researchers were able to find out if the drug was doing any good in just four to eight weeks. Liquid biopsies are also a promising technology for the routine monitoring of patients who have been successfully treated for cancer, lest their disease return. Mark Roschewski, a researcher with America's National Cancer Institute, the NCI, thinks the technology could be "orders of magnitude more sensitive than radiographic imaging".

Biopsies optimised

The big question for the firms developing these liquid biopsies is whether the technology will also be suitable for the early-detection market that Mr Boyle is chasing with his breath tests. Guardant Health, a firm based in Redwood City, California, currently offers a liquid biopsy that allows patients to obtain a genetic profile of their tumour. It is using the data it gathers to look at the feasibility of early detection. Helmy Eltoukhy of Guardant says the firm is "agnostic" about the markers it seeks in the blood, meaning that its researchers will not look just for DNA from tumours—if the data suggest that RNA (a relative of DNA) or proteins provide the telltale fingerprint, then that is what they will look at.

All diagnostic tests have to overcome two hurdles. They have to be sensitive enough to identify those who have the disease correctly and also specific enough that they do not see signs of the disease when it isn't actually present. The more widely they are used, the more important that second requirement gets; false positives are a pervasive problem with existing tests such as mammograms and PSA, a test for prostate cancer. (This is why PSA screening, while common in America, is much less prevalent in Europe.)

In liquid biopsies the challenge will be to detect cancer-specific signals against a noisy and confusing background. Barry Kramer, director of the division of cancer prevention at the NCI, warns that the same marker can have different functions in different organs. He notes that a programme screening infants for neuroblastoma was halted after it started to pick up too many growths that did not merit clinical concern and didn't reduce the death rate. Specificity, says Mr Eltoukhy, is early detection's Achilles' heel. Others warn that liquid bi-



Improved diagnostics reveal cancer's weaknesses



DNA will never be sensitive enough for early de-
early tumours may shed very little DNA, or shed it
ly; other molecules might prove more telling.

biotech is gung-ho about the idea. Grail, a liquid-
n Silicon Valley spun out of Illumina, a sequencing
aised \$900m. Earlier this year Guardant raised
habet invested \$65m in Freenome, a San Francisco
nilar plans. Grail has begun a trial of its technology
oll 120,000 women who are receiving mammo-
ts technology really does offer early detection.

will make sense to adapt liquid biopsies to popula-
will depend on their costs—currently still too high
use—their sensitivity and, crucially, their false-pos-
necessary investigations after false positives are
; and debilitating for patients and costly for the
tem. But some, such as Luis Diaz, an oncologist at
n Kettering, argue that initial overdiagnosis is a nec-
moving ahead. "One never learns to ride a bike
off."

nd difficulties of blood screening are one of the
y Owlstone has going for it. Testing the breath for
esn't require the tumours to have started shedding
NHS is running a £1m trial of the technology in pa-
d of having lung cancer who are also being exam-
means. If this finds the technology to be reliable it
ned for use in population screening. In July Owl-
ould collaborate with academic partners to see if
s could be expanded to pick up bladder, breast, kid-
n, prostate, brain, and head and neck cancers.

question that blood biopsies will be at the heart of
racking and profiling tumours. But for early detec-
tions might yet win out; success will not hinge on
ny starts with the most money but which offers the
or the buck. Health-care systems will seek to adopt
that work at scale. The benefit will be that more can-
red with the most basic, oldest and most effective
ncer treatment. ■

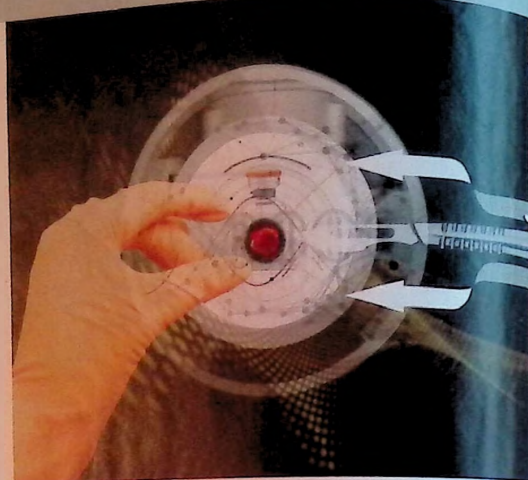
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ression, and how p it

otherapy and cancer drugs are all becoming
ocused

MILLEY'S primary tumour was in the middle lobe
her right lung, which surgeons removed entirely.
urgery is an ancient form of cancer treatment and
ll a common one. Today's surgeons have every-
ing from lasers to cryosurgery—the freezing of ab-
normal tissue—at their disposal. By and large, they
ding range of tools to cut out less and less. Ultra-
tic-resonance imaging (MRI), x-ray tomography
mission tomography (PET) scans have between
ed much of the need for "exploratory surgery" to
scope of a cancer.

y goes hand-in-hand with radiation therapy. Soon
ery of x-rays at the end of the 19th century it be-
radiation which killed cells could be used as a can-
early days practitioners judged the correct dose
machines out on their own arms, looking for a pink
r skin. Many went on to develop leukaemia.



Today radiotherapy is considerably safer for its practitioners
and more beneficial to its recipients. After a cancer is cut out, radia-
tion is frequently used to kill the cancer cells the surgeon's knife
has missed. It is also sometimes used to destroy the tumours
themselves, particularly in places where surgery would be hard.
In rich countries about half of patients with localised cancers re-
ceive radiotherapy. Two out of five of those treated for cancer and
cured in Britain will have had treatment which consisted of radio-
therapy either alone or in part. Breast and prostate cancers re-
spond well to it.

To make all this possible, medical physicists produce beams of
x-rays, gamma radiation, neutrons and, increasingly, protons;
they have ever more sophisticated ways of ensuring that these
cell-damaging energies are delivered to the tumours being target-
ed, rather than to healthy tissue nearby. Ms Milley experienced
this when she had a superficial brain metastasis dealt with by ste-
reotactic radio-surgery. The procedure uses 3D imaging to deter-
mine the exact location of a tumour, at which point a number of
different beams are focused on it from various directions (see dia-
gram on next page). The idea is that only in the part of the brain
where all the beams cross is the dose high enough to kill cells—the
individual beams, on their way in and out, do comparatively little
damage. The idea is to match the extent of the lethal criss-crossing
as closely as possible to the location of the tumour. It is a way of
achieving what Emma Harris, a medical physicist with the Insti-
tute of Cancer Research (ICR) in London, calls the current state of
the art: "Shaping the beam and varying the intensity of the radia-
tion dose to create exquisite volumes of radiation."

Proton therapy offers another way to deal death to tumours
while sparing the surrounding tissues. By choosing the right ener-
gy for the beam physicists can determine how deep into the tissue
it will get before doing most of its damage. This specificity is seen
as particularly useful in tumours that are near eyes, brains and spi-
nal cords.

Radiation can also be emitted inside the body; radioactive pel-
lets and seeds can be put right where they are needed. A new ver-
firm based in Paris, which is developing nanoparticles containing
hafnium oxide which generate electrons when exposed to x-rays.
When these nanoparticles are injected into tumours that are then
zapped with x-rays they increase the damage done.

As well as surgery on the lung and radiation treatment for the
tumour in her brain, Ms Milley also had chemotherapy—the third
of the 20th century's medical responses to cancer. She was given a
cocktail of cisplatin, a drug containing platinum that was ap-

proved in 1978, and Alimta (pemetrexed).

Chemotherapy's origins can be traced
back to the development of chemical
weapons in the first world war. Looking
into the records of soldiers affected by
mustard gas, two doctors at Yale Universi-
ty, Louis Goodman and Alfred Gilman, no-
ticed that many were short of white blood
cells. They wondered if this meant that
cancers in which white blood cells prolifer-
ate—lymphomas—might be treated with
something similar. The first patient to re-
ceive this treatment was a man with ad-
vanced lymphoma who is known today
by the initials "J.D.". His symptoms were
greatly relieved.

The treatment worked because mustard gas damages cells'
DNA, stopping cell division. These effects are not specific to cancer
cells; but because cancer cells divide a lot, such poisons are particu-
larly bad for them. In 1947 aminopterin, a chemical which
messes up cell division by interrupting the metabolism of folic
acid, was found to produce remissions in children with acute leu-
kaemia. This drug was a precursor to methotrexate, a treatment
which provided the first cures of a metastatic cancer in 1956 and is
still commonly used today. By the 1960s, chemotherapy had in-
duced long-term remissions, and even cures, of Hodgkin disease,
a lymphoma, and childhood acute lymphoblastic leukaemia.
Cures of testicular cancer arrived in the 1970s. Though few cancers
can be cured with chemotherapy on its own, many can be set back
a long way and controlled for quite some time. Chemotherapies,
like radiation therapies, are often used to mop up the cancer left
over when primary tumours have been excised.

Elective affinities

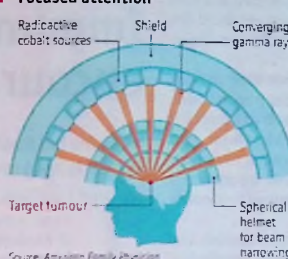
One problem with chemotherapy is that cancers can become re-
sistant to it. Most cancers are genetically heterogeneous, because
the cells accumulate new mutations as they grow. Some of these
mutations can make the cells less susceptible to the chemothera-
py. As treatment continues, such cells become more numerous,
and as they divide they go on to accumulate mutations that make
them even more resistant—the cancer evolves resistance to che-
motherapy rather than an infection can evolve resistance to antibiot-
ics. This is why chemotherapies are now often used in combina-
tion; it is harder to evolve resistance to two or three drugs at the
same time.

Another problem with chemotherapy is that it attacks cells that
are dividing for perfectly legitimate non-cancerous reasons, too.
Hence the side-effects, which include fatigue, hair loss, mood
changes and nausea. The severity of the effects vary greatly from
person to person, and some, such as nausea, can be treated with
secondary drugs under some circumstances. But some chemo-
therapies can have long-term side-effects, damaging the heart, the
nerves and fertility.

Before taking on her chemotherapy, though, Ms Milley was giv-
ing another treatment: Tarceva (erlotinib). Tarceva is a small mole-
cule which disrupts signals transmitted by a protein called the epi-
dermal growth-factor receptor (EGFR). At least eight mutations
that cause the EGFR to be constantly active have been tied to lung
cancer, and Ms Milley had one of them. Her course of Tarceva saw
all the tiny tumours across her body shrink, one by 60%. She went
back to work.

A key tool for targeting cancer-specific pathways and mole-
cules is the antibody. Antibodies are proteins made by the im-
mune system which stick to a particular bit—the "antigen"—of a
particular molecule. Turning them into mass-produced drugs has
been one of the biotech industry's triumphs. In the 1990s they
started to come into use as cancer therapies. Aimed at antigens
that crop up on cancers, but not other cells, they are far more spe-

Focused attention



cific than older chemotherapies. Rituxan
(rituximab), an antibody which targets a
protein on the surface of the immune sys-
tem's B-cells that misbehaves in B-cell non-
Hodgkin lymphoma, was approved 20
years ago, in 1997. Other early targeted ther-
apies that blocked growth signals in differ-
ent cancers included Herceptin (trastuzu-
mab) and Erbitux (cetuximab), which are
both antibodies, and Iressa (gefitinib) and
Gleevec (imatinib), which are smaller mol-
ecules like Tarceva. These drugs trans-
formed the treatment of many cancers.
Herceptin, for example, dramatically al-
tered the outcome of breast cancer in pa-
tients with the HER2 mutation. With Her-
ceptin as part of a two-drug therapy, a woman diagnosed with the
metastatic form of the disease can hope to survive for almost five
years; previously it was 20 months.

Another promising targeted approach involved aiming drugs
at the creation of new blood vessels. If tumours are to grow be-
yond a few millimetres in size they need to encourage new blood
vessels to bring them nutrients. Drugs which inhibit this process
arrived in 2004 with Avastin (bevacizumab). It is currently used to
treat advanced colorectal, kidney and lung cancers.

A third approach attacks DNA repair systems. Losing some of
the ability to repair DNA helps cancers accumulate mutations, and
is often part of how they get started. But the cancers need to keep
some residual DNA repair functions; otherwise the cells will sim-
ply die. Thus cancers that have mutations in the BRCA1 and BRCA2
genes rely heavily on a backup DNA repair mechanism which
uses proteins called poly-ADP-ribose polymerases (PARPs). Now
targeted drugs have been designed to inhibit this repair mecha-
nism. In its absence, massive genetic damage drives cancer cells to
their death. Some of these PARP inhibitors have been shown to
help in BRCA-linked breast cancers, and there are promising re-
sults in ovarian cancer. They seem also to have promise in some
prostate and pancreatic cancers.

Finding targets for such therapies has been made far easier by
the sequencing of the human genome and the remarkable reduc-
tions in the cost of sequencing DNA which followed on from it.
With a baseline genome for comparison, identifying the muta-
tions in cancers became much easier. Once found, these genes can
be used to understand the molecular workings of the disease and,
in theory, to find new targets for drug developers.

Imperfect chemistries

Mike Stratton, director of the Sanger Institute, set up its cancer-ge-
nome project in 2000, when sequencing was still a comparatively
arduous business. They were interested in looking at mutations of
40 different genes, but practical limitations meant they could only
start working on 20. The third gene they looked at was BRAF; se-
quencing the genes from 500 cancers the researchers found that
there were BRAF mutants putting yet another cell-growth-signal-
ing pathway into overdrive in half or more of the malignant mela-
nomas in their sample. By 2011 the first BRAF inhibitor, Zelboraf
(vemurafenib), was approved for the treatment of melanoma. In a
trial, the six-month survival was 84%, compared with 64% who
were treated with chemotherapy. The drug was quite toxic—de-
spite their targeting, such drugs do have side-effects—but it was still
approved for use.

Hundreds of thousands of cancers have now been sequenced,
and the hunt for targets is seeing diminishing returns. Though
there are hundreds of genes that go wrong in cancers, only a lim-
ited number promote cancer development and are common to a
number of cancers. A significant amount of work is now focused
not on finding new targets but on second-generation drugs aimed
at targets that have already proved vulnerable; these newer drugs ►

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when they treated both cell cultures and laboratory animals with GSK2816126, they found it also reduces the proliferation of tumour cells while, crucially, having no apparent effect on nearby normal cells.

James Bradner of the Dana-Farber Cancer Institute, in Boston, described a second epigenetic approach to treating cancer. His group have shown that a substance known as JQ1, which inhibits an epigenetic regulator called BRD4, blocks the activity of a gene by the name of Myc. Myc encodes a protein called a transcription factor that is another part of the DNA-regulation system. This particular transcription factor is involved in the expression of about 15% of human genes. Not surprisingly, then, when it goes wrong it is one of the most common causes of cancer.

Despite numerous attempts, researchers have been unable to find a way to block the activity of Myc directly. Dr Bradner, however, reasoned that blocking BRD4, which is one of Myc's collaborators, might do the job indirectly. To test this thought he and his colleagues treated mice suffering from Myc-driven myeloma with JQ1. And it worked. JQ1, they found, shut down Myc-activated genes and slowed the proliferation of myeloma cells.

Although neither GSK2816126 nor JQ1 is ready for human trials, two other sorts of epigenetic drugs are already on the market. DNA-demethylating agents, in the form of azacitidine, sold as Vidaza by Celgene, of Summit, New Jersey, and decitabine, sold as Dacogen by Eisai, a Japanese company, are used to treat myelodysplastic syndromes, the precursors of acute myelogenous leukaemia. And histone-deacetylase

TECHNOLOGY QUARTERLY | Treating cancer

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But there are still new targets being hit for the first time. In 2016, the drug Xalkori (crizotinib) was approved for ROS1-positive lung cancer. Louis Staudt, director of the centre for cancer genomics at the National Cancer Institute (NCI) in Bethesda, Maryland, says about 1-3% of cases of lung cancer are driven by a ROS1 mutation. Dr Staudt is working on a repository for genomic information called the NCI Genomic Data Commons, which hopes to identify more low-frequency drivers of cancer.

These targeted therapies are changing the way the doctors and regulators look at cancer. Typically cancers have been classified according to where they occur and how they behave. Now they can also be classified according to which genes are going wrong in them. This allows new sorts of investigation such as the NCI's MATCH trial, which matches patients to treatments based on the genetic changes in their tumours. More than 6,000 patients treated at more than 1,000 institutions have had their tumours sequenced as part of this trial. The large numbers are needed to pick out the rare mutations that drive cancers.

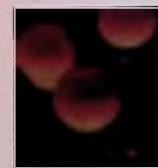
By their genes shall you know them

A milestone in the transition to a genomic era for cancer therapy was reached earlier this year when America's Food and Drug Administration (FDA) approved a treatment based on a specific genetic indicator rather than the type of tumour, as determined by location and its tissue structure. A similar "biomarker"-based approval is expected soon for a drug which targets a defect in a family of signalling proteins called tropomyosin receptor kinases, proteins which play an important role in tumour growth. A rare mutation (it affects only about 1% of patients) sees the genes that code for TRKs become fused to other genes. Loxo Oncology, a biotechnology firm in Stamford, Connecticut, has developed a drug aimed at this aberration: the idea is that it should be licensed for use in anyone with the relevant mutation.

Targeted therapies mark a significant advance over, and addition to, older chemotherapies. But they share their familiar weaknesses. In the 2000s Olivia Rossanese, a researcher at the ICR, worked on a BRAF inhibitor at the British pharma firm GSK. She says: "We made a drug to it, we said patients with this mutation are going to respond and that happened. It was a beautiful story...right up until resistance." To cancers, targeted therapies, including antibodies, are another constraint to evolve around, and in the end that is what they tend to do.

For 13 months Ms Milley responded wonderfully to Tarceva. Then her doctor at the Dana-Farber Cancer Institute in Boston noticed spots on her adrenal glands that indicated resistance. That was the point at which she started cisplatin chemotherapy. It worked for eight months. When it started to falter, she went back on to the Tarceva, which worked for another four months. That looked like the end of the road for approved treatments. The only remaining option seemed to be to take a chance with a clinical trial, and this she agreed to do.

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"It was a beautiful story...right up until resistance"

Loosening inhibitions

Immunity, and how to encourage it

Medicine has finally figured out how to get the immune system to fight cancer

THAT infections could sometimes cause a cancer to retreat, or even vanish, was known well before the advent of modern medicine. Imhotep, a pharaoh, recommended treating a tumour with a poultice followed by an incision—something that would help an infection develop. In early modern Europe doctors used septic dressings on tumours with ulcers and deliberately created purulent sores. By the end of the 19th century, William Coley, a bone surgeon in New York, was methodically infecting patients with *Streptococcus* bacteria.

Coley's work fell out of favour, partly thanks to the rise of radiation therapy. Many continued to cling to the idea that the immune system might in some circumstances be provoked into recognising, attacking and subduing a cancer; they just didn't know how to provide the provocation. In 1976 this latent belief in the potential of "immunotherapy" blossomed into hope with the discovery of interleukin 2 (IL-2). IL-2 is a growth factor that encourages the production of T-cells, white blood cells that scan the body for unwanted invaders and, on finding them, activate other parts of the immune system, including the B-cells which produce antibodies.

But IL-2 was a false dawn. On its own, it activated the immune system indiscriminately, and the immune system is a powerful beast; Elad Sharon, at the National Cancer Institute's division of treatment and diagnosis, says the effects were "toxic and messy, and frequently sent patients to the ICU." That might have been more tolerable in a drug that delivered. But to general surprise and discouragement IL-2 cured only a few patients of metastatic cancers. It was not clear why the treatment was not more effective.

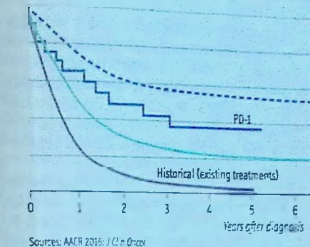
Answers started to arrive in the 1990s. James Allison, at the Cancer Research Laboratory at the University of California, Berkeley, began work on a protein called CTLA-4 on the surface of some T-cells. By 1996 he had shown that this protein put a brake on the immune response to cancer. Blocking CTLA-4 with an antibody removed the brake; the immune system activated itself and got to work. Tumours in mice vanished when the animals were given CTLA-4-blocking antibodies. Though it was not immediately obvious, in retrospect this came to be seen as one of the reasons IL-2 never really worked: it is not possible to make a car run faster if its brakes are jammed on.

At the time oncologists were unimpressed by Dr Allison's results. Cancer had been cured in mice many times over. And after many failed trials, immunotherapy was in exile—banished to the small corners of the big oncology meetings. But in 1999 Tasuku Honjo of the University of Kyoto, in Japan, showed that the gene for a protein called PD-1 also seemed to tamp down the immune system. When this gene was switched off in mice, some developed autoimmune diseases—a sign of an immune system in overdrive. In collaboration with Arlene Sharpe and Gordon Freeman at Harvard, Dr Honjo showed that some cancer cells had a second protein called PD-L1 on their surfaces which, by interacting with the PD-1 on T-cells, protected the cancer from them. Dr Honjo remembers approaching many companies with the finding, but "none wanted to invest."

Despite a general wariness on the part of pharma companies, though, a trickle of development on therapies aimed at CTLA-4 and PD-1 did begin. Then, in 2010, Bristol-Myers Squibb released results from a trial of an anti-CTLA-4 antibody, Yervoy (ipilimumab).

Stepping up

Survival rates of melanoma patients, by type of treatment, %



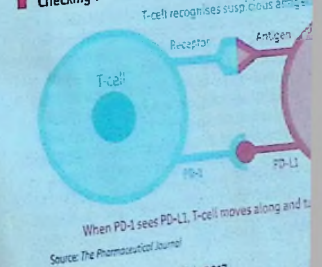
mab), in malignant melanoma. Compared with the art, they were fantastic. It was the first drug shown to rival in this devastating disease, raising the median time Some survived much longer.

What was going on? Because the immune system is a powerful beast, evolution has equipped it with a system and balances lest it get out of hand. Both CTLA-4 and PD-1 are part of that system. When one sort of immune cell presents an antigen to another, the second cell will kill it. At the same time, the first cell stimulates the CTLA-4 receptor. CTLA-4 receptor is blocked with an antibody like Yervoy. This "checkpoint" system does not work. Unchecked, the immune system is able to react to a wider range of antigens than it should. Freed up by Yervoy the body's immune system attacks the melanomas. And, it turned out, kept them. Perhaps the most remarkable feature of the "point inhibitor" was that a small subset of patients survived for years.

Despite indications of success with melanoma, many doctors thought the checkpoint-inhibitor mechanism was broadly effective. Melanomas accumulate a very high number of mutations, and are thus more likely than most cancers to trigger an immune response. This is bolstered by the observation that melanomas are more likely than other cancers to be subject to spontaneous remission, because something else kicks the immune system into action. What was more, Yervoy had serious, sometimes life-threatening side-effects.

Pessimists have a pretty good record when it comes to prognostication. But this time they were wrong. Perlmutter, an immunologist who had previously worked at the Dana-Farber Cancer Institute, was brought back to run the research labs. He was interested in a PD-1 blocker then known only as BMS-936233, which works higher up the immune system than CTLA-4, which has a front-line role; if a cancer

Checking out



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Genes shall you know them

Stone in the transition to a genomic era for cancer therapy. The FDA approved a treatment based on a specific gene, imatinib, rather than the type of tumour, as determined by its tissue structure. A similar "biomarker"-based approach is expected soon for a drug which targets a defect in a family of proteins called tropomyosin receptor kinases, proteins which play an important role in tumour growth. A rare mutation affects only about 1% of patients. The genes that code for these proteins are fused to other genes. Loxo Oncology, a biotech firm in Stamford, Connecticut, has developed a drug targeting this aberration; the idea is that it should be licensed for use with the relevant mutation.

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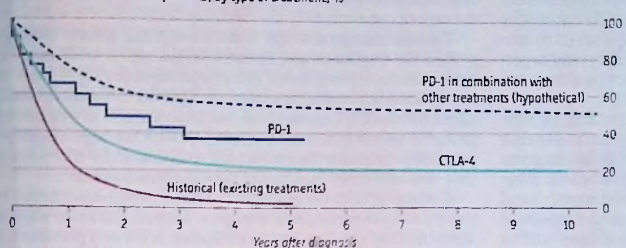
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The Economist September 16th 2017

Stepping up

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Sources: AACR 2016; J Clin Oncol

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What was going on? Because the immune system is such a powerful beast, evolution has equipped it with a system of checks and balances lest it get out of hand. Both CTLA-4 and PD-1 are parts of that system. When one sort of immune cell presents an antigen which it has picked up to another, the second cell will ignore it if, at the same time, the first cell stimulates the CTLA-4 receptor. If the CTLA-4 receptor is blocked with an antibody like Yervoy, though, this "checkpoint" system does not work. Unchecked, the immune system is able to react to a wider range of antigens—including tumour antigens. Freed up by Yervoy the body's T-cells started attacking the melanomas. And, it turned out, kept on attacking them. Perhaps the most remarkable feature of the new "checkpoint inhibitor" was that a small subset of patients survived for year after year.

Despite indications of success with melanomas, many scientists thought the checkpoint-inhibitor mechanism would not be broadly effective. Melanomas accumulate a very large array of mutations, and are thus more likely than most cancers to display antigens which trigger an immune response. This argument was bolstered by the observation that melanomas are more likely than other cancers to be subject to spontaneous remissions—presumably because something else kicks the immune system into gear. What was more, Yervoy had serious, sometimes life-threatening side-effects.

Pessimists have a pretty good record when it comes to cancer prognostication. But this time they were wrong. At Merck Roger Perlmutter, an immunologist who had previously left the company, was brought back to run the research labs. He became very interested in a PD-1 blocker then known only as MK-3475. Unlike CTLA-4, which works higher up the immune system's chain of command, PD-1 has a front-line role; if a cancer cell carries PD-1's

counterpart, PD-L1, on its surface, T-cells will ignore the cell despite any suspicious antigens it may be carrying (see diagram). MK-3475 seemed to block the interaction nicely. It might thus render the immune system blind to the cancer's subterfuge. "Whatever [else] you are doing, stop," Perlmutter told his clinical-development group. Merck expanded a phase 1 trial programme looking at the drug's effect on advanced melanoma to more than 1,200 patients, making it the largest phase 1 trial in the history of oncology.

The expansion was in part a response to a new discovery: early evidence suggested that checkpoint inhibitors could also get results with lung cancers, which are a much

bigger killer than, and thus represent a much larger market than, malignant melanomas. Luis Diaz, head of solid-tumour oncology at the Memorial Sloan Kettering Cancer Centre in New York, recalls: "It was completely unexpected. Prior to that I was not a believer in immunotherapy."

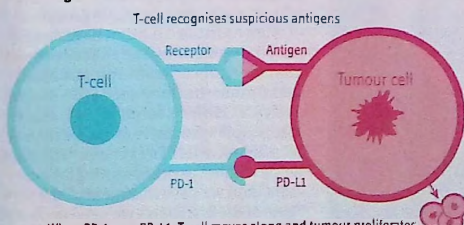
Whispers of a cure

Merck's PD-1 drug would eventually be given the commercial name Keytruda (pembrolizumab). In 2014 it became the second checkpoint inhibitor to be approved in America—the world's largest and most lucrative pharmaceutical market. Opdivo (nivolumab), a PD-1 drug which Ono Pharmaceuticals had developed on the basis of Dr Honjo's work, soon joined it, having been licensed in Japan a little earlier. In some cases the drug produced effects little short of miraculous. In 2016 it was announced that it had cleared former president Jimmy Carter of metastatic melanoma that had spread to his liver and brain.

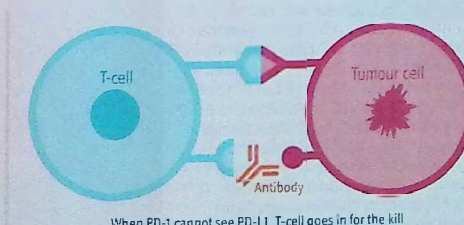
In lung cancer, and in many other cancers, the patients who responded tended to have a higher mutational burden, like that seen in melanoma. More antigens means more targets for the immune system to tackle when the drug lets it off the leash. This observation provided a way to spot some of the patients most likely to benefit. In 2017 Keytruda was approved for use in any cancer that has mismatch-repair-gene defects, a flaw which means that a cancer accumulates even more mutations—and thus more possible antigens—than most.

Another indicator that the drug may have something to offer is the tumours' expression of PD-L1. Tumours expressing a lot of PD-L1 are investing in keeping the immune system duped; when the PD-1 system is interrupted they should prove particularly vulnerable. At the start of October 2015, Keytruda was approved for use in advanced non-small-cell lung cancer in cases where other treatments had failed and when there was PD-L1 on more than 50% of the tumour cells. Ms Milley's score was 80%, and she started treatment almost immediately.

Checking out



Source: The Pharmaceutical Journal



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Despite numerous attempts, researchers have been unable to find a way to block the activity of Myc directly. Dr Bradner, however, reasoned that blocking BRD4, which is one of Myc's collaborators, might do the job indirectly. To test this thought he and his colleagues treated mice suffering from Myc-driven myeloma with JQ1. And it worked. JQ1, they found, shut down Myc-activated genes and slowed the proliferation of myeloma cells.

Although neither GSK2816126 nor JQ1 is ready for human trials, two other sorts of epigenetic drugs are already on the market. DNA-demethylating agents, in the form of azacitidine, sold as Vidaza by Celgene, of Summit, New Jersey, and decitabine, sold as Dacogen by Eisai, a Japanese company, are used to treat myelodysplastic syndromes, the precursors of acute myelogenous leukaemia. And histone-deacetylase

TECHNOLOGY QUARTERLY | Treating cancer

Jedd Wolchok, a medical oncologist at Memorial Sloan Kettering, says immunotherapies do not have the same kinds of impact as other types of cancer therapy. In some cases they do not work at all. In other cases they can either eliminate the cancer entirely, or cause it to stabilise, or regress. Responses to therapy are often longer lasting than those seen in targeted drugs. And they tend to persist after patients stop taking the drug (at present CTLA-4 drugs are usually administered for only a matter of months).

The nature of the long-lasting responses is intriguing. Dr Wolchok has patients who started treatment for malignant melanoma eight years ago. He finds it particularly interesting that in some cases scans of the cancers taken before treatment (when the prognosis for the patients would have been six or seven months) and scans taken today look more or less equally dreadful. Biopsies of the tumours reveal a lot of immune cells and a lot of dead tumour cells. Dr Wolchok says it looks like a "chronic struggle between a patient's immune system and cancer". This apparent equilibrium

is quite different from what is seen in chemotherapy, where the cancer will be either susceptible or resistant. The difference seems to be due at least in part to the fact that the immune response, like the cancer, can evolve.

Though immunotherapy is still new, it has already radically shifted the treatment and research landscape. A wide range of combinations is being tested in the hope of improving patients' responses. A trial combining Opdivo and Yervoy in malignant melanoma has shown tumours to shrink in 60% to 70% of patients (although it causes serious side-effects). Dr Wolchok says it is not yet possible to calculate the median survival time in the trial population—because more than half of the patients are still alive.

Compared with the more limited range of patients that can be treated with most targeted therapies, immunotherapies seem to work in many cancers. And as Dr Sharon at the NCI points out, it also produces cures. But this excitement needs to be tempered with the grittier reality that, across all cancers tried so far, only

Brain scan | Meeting the unmet burden

Much of the world has yet to see progress on cancer

MEG O'BRIEN, the director of global cancer treatment at the American Cancer Society (ACS), first went to Africa some 15 years ago to work on HIV. She has thus seen at first hand how advanced treatments can make a difference to deadly diseases in poor countries. Now she wants to see the same done for cancer. But it is an uphill struggle.

It is not just that the resources needed are not there. Nor is the hope. "Nobody in Africa knows anyone who survives cancer," she says. "It is considered 100% fatal and is associated with so much suffering." This means that the idea of getting tested for cancer when symptoms are spotted is a hard sell. Indeed, according to Dr O'Brien, there is more stigma around cancer in parts of Africa than there is around AIDS.

Working with IBM, the multinational technology firm, Dr O'Brien has been trying to improve the availability of cancer treatments in sub-Saharan Africa. She says the biggest barrier is that the market for pharmaceuticals functions so poorly because of a set of interlocking problems—what she calls a "twelve-part equation".

Because there is very little cancer treatment at present the agencies which procure medicines are unfamiliar with the cancer treatments they might want; their interests have mostly been focused on infectious disease. The pharmaceutical companies, for their part, neglect the market because of the low volumes—low volumes which mean that the procuring agencies pay higher prices than they should for old, off-patent drugs. Countries are often inundated by counterfeit

products (sometimes taking as much as 40% of the market) and many of the products being sold would never be approved by a stringent regulator.

The ACS and IBM have been working on ways to predict the number of cancer cases countries can expect to see. That should allow drugs to be ordered in bulk at wholesale prices from reliable suppliers. The ACS is also helping countries redraft their guidelines for treating cancer so that they match the locally available treatments.

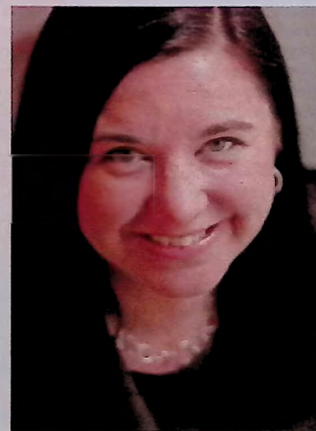
The effort extends to private pharmacies. At the moment their incentives encourage them to buy either the products with the highest margins or those that sell for the least. The charity is devel-

oping a tool called "chemo finder" that doctors can use to find pharmacies that have high-quality products at competitive prices. Dr O'Brien hopes that this tool will encourage pharmacies to bring in the products that the doctors most need.

In Uganda another innovation is to create partnerships between cancer-care centres and experts abroad who can diagnose pathology slides that have been uploaded into the cloud.

Such progress is urgently needed, because the number of patients with cancer in low- and middle-income countries is rising rapidly. BIO Ventures for Global Health (BVGH), a non-profit organisation based in Seattle, Washington, notes that 60% more Africans die from cancer than succumb to malaria. A white paper from BVGH says that over 20% of African countries have no access to cancer treatments at all and most cancers are diagnosed at an advanced stage. The five-year breast cancer survival rate in the Gambia is 12%, compared with 90% in America. The number of deaths is rising at an alarming rate, thanks to ageing and the spread of Western lifestyles. In some countries the proportion of the population that smokes tobacco is growing as people get richer.

Many African countries have rapidly growing economies. They already spend money on cancer treatments and could spend more—but they need to do so effectively. Dr O'Brien's emphasis is to make sure people who arrive at cancer centres get a decent level of treatment. "Right now we have more patients waiting for treatment than we have treatments," she says. "One of the first things we need to do is get drugs on the ground."



about 20% respond to the new approach. The greatly between types of cancer. In patients with usual treatments for Hodgkin's lymphoma it is 90% and most colorectal cancers it is basically zero.

Improving this response is perhaps today's biggest challenge—the source of more excitement, and many other recent developments in the field. Part of it comes from a better understanding of the steps needed for an anti-tumour response from the immune system. Therapeutic targets available. For example, Hervé Delacourte, boss of biotech firm Incyte, a biotech firm based in Delaware, says that some tumours protect themselves from the immune system using another checkpoint, PD-1. PD-1 was first discovered in a search for ways to protect immune rejection. Incyte is testing epacadostat, a known to inhibit PD-1, as a cancer treatment in combination with PD-1 blockers.

There are well over 1,000 clinical trials of checkpoint inhibitors going on; what was at first a trickle, then a current. Some worry that things have gone too far too fast. Dr Freeman, who runs the new Parker Institute for Cancer Immunotherapy in San Francisco, says "many of [these trials] are based on data and very limited clinical evidence about whether they will work". Some fear there are too few patients in trials to be run, others that there is too little thought and a lot of duplication of effort. Dr Freeman at has been told there are over 80 Chinese groups doing PD-1 antibodies.

This enthusiasm may lead to wasted efforts, progress. But there is no doubt that immunotherapy here on be a key part of treatment for a growing number of cancers. Perhaps the most telling measure of its success oncologists have started to complain, quietly, of a shortage of specialist doctors. Patients keep coming back instead

Building on success

The future, and how to get there

There is a lot more for immunotherapy to do

IMMUNOTHERAPY offers huge promise. It has led to established therapies and as a foundation for new ones. Hundreds of trials are pairing CTLA-4 inhibitors with chemotherapy, radiation and surgery. One hope is that the older treatments will be replaced by immunotherapy. The range of antigens that the cancer offers the immune system to latch on to, both by driving further immune response and by killing cancer cells. Dead cells release more antigens for the immune system to latch on to.

Then there is the development of further immunotherapies, which is being pursued both by building on the existing ones and by using entirely new approaches. The first checkpoint inhibitors and by using entirely new approaches, such as genome editing. Dr Wolchok at Memorial Sloan Kettering is working on the next generation of immune-mimicking compounds for PD-1 and CTLA-4. Others include new inhibitory compounds for TIM-3 and VISTA. Some researchers are trying to remove the immune system's brakes by silencing some of its checkpoints. Others are looking at molecules which activate the immune system in a similar way to 11-2. Nektar Therapeutics, based in San Francisco, is developing an enzyme-based immunomodulator, which does this in a way that should, in principle, be more effective than existing drugs.

Wolchok, a medical oncologist at Memorial Sloan Kettering, says immunotherapies do not have the same kinds of impact as chemotherapy. In some cases they do not work at all, in others they can either eliminate the cancer entirely, or stabilise, or regress. Responses to therapy are often longer than those seen in targeted drugs. And they tend to persist after patients stop taking the drug (at present CTLA-4 drugs are administered for only a matter of months).

One of the long-lasting responses is intriguing. Dr Wolchok says that patients who started treatment for malignant melanoma a year ago. He finds it particularly interesting that in some cases the cancers taken before treatment (when the prognosis would have been six or seven months) and now today look more or less equally dreadful. Biopsies of the tumours reveal a lot of immune cells and a lot of dead tumour cells. Dr Wolchok says it looks like a "chronic struggle between an immune system and cancer". This apparent equilibrium

is quite different from what is seen in chemotherapy, where the cancer will be either susceptible or resistant. The difference seems to be due at least in part to the fact that the immune response, like the cancer, can evolve.

Though immunotherapy is still new, it has already radically shifted the treatment and research landscape. A wide range of combinations is being tested in the hope of improving patients' responses. A trial combining Opdivo and Yervoy in malignant melanoma has shown tumours to shrink in 60% to 70% of patients (although it causes serious side-effects). Dr Wolchok says it is not yet possible to calculate the median survival time in the trial population—because more than half of the patients are still alive.

Compared with the more limited range of patients that can be treated with most targeted therapies, immunotherapies seem to work in many cancers. And as Dr Sharon at the NCI points out, it also produces cures. But this excitement needs to be tempered with the grittier reality that, across all cancers tried so far, only

In scan | Meeting the unmet burden

The world has yet to see progress on cancer

RIEN, the director of global treatment at the American Cancer Society, first went to Africa some 15 years ago to work on HIV. She has thus learned how advanced treatment makes a difference to deadly diseases. Now she is the same done for cancer.

With the resources needed to fight the disease. "Nobody in the world is anyone who survives cancer," she says. "It is considered 100% associated with so much stigma that the idea of dying for cancer when symptoms are a hard sell. Indeed, accordingly, there is more stigma in parts of Africa than in the West."

With IBM, the multinational pharmaceutical firm, Dr O'Brien has been able to improve the availability of treatments in sub-Saharan Africa. The biggest barrier is that the pharmaceutical functions so much of a set of interlocking interests that she calls a "twelve-part

There is very little cancer research in the agencies which are unfamiliar with treatments they might want; they have mostly been focused on diseases. The pharmaceutical part, neglect the market for low volumes—low volume means that the procuring of higher prices than they can pay for off-patent drugs. Countries are inundated by counterfeit

products (sometimes taking as much as 40% of the market) and many of the products being sold would never be approved by a stringent regulator.

The ACS and IBM have been working on ways to predict the number of cancer cases countries can expect to see. That should allow drugs to be ordered in bulk at wholesale prices from reliable suppliers. The ACS is also helping countries redraft their guidelines for treating cancer so that they match the locally available treatments.

The effort extends to private pharmacies. At the moment their incentives encourage them to buy either the products with the highest margins or those that sell for the least. The charity is devel-



oping a tool called "chemo finder" that doctors can use to find pharmacies that have high-quality products at competitive prices. Dr O'Brien hopes that this tool will encourage pharmacies to bring in the products that the doctors most need.

In Uganda another innovation is to create partnerships between cancer-care centres and experts abroad who can diagnose pathology slides that have been uploaded into the cloud.

Such progress is urgently needed, because the number of patients with cancer in low- and middle-income countries is rising rapidly. BIO Ventures for Global Health (BVGH), a non-profit organisation based in Seattle, Washington, notes that 60% more Africans die from cancer than succumb to malaria. A white paper from BVGH says that over 20% of African countries have no access to cancer treatments at all and most cancers are diagnosed at an advanced stage. The five-year breast cancer survival rate in the Gambia is 12%, compared with 90% in America. The number of deaths is rising at an alarming rate, thanks to ageing and the spread of Western lifestyles. In some countries the proportion of the population that smokes tobacco is growing as people get richer.

Many African countries have rapidly growing economies. They already spend money on cancer treatments and could spend more—but they need to do so effectively. Dr O'Brien's emphasis is to make sure people who arrive at cancer centres get a decent level of treatment. "Right now we have more patients waiting for treatment than we have treatments," she says. "One of the first things we need to do is get drugs on the ground."

about 20% respond to the new approach. The response varies greatly between types of cancer. In patients who have failed the usual treatments for Hodgkin's lymphoma it is 90%. In pancreatic and most colorectal cancers it is basically zero.

Improving this response is perhaps today's biggest oncological challenge—the source of more excitement, and investment, than any other recent development in the field. Part of the answer will come from a better understanding of the steps needed to generate an anti-tumour response from the immune system, and of the therapeutic targets available. For example, Hervé Hoppenot, the boss of biotech firm Incyte, a biotech firm based in Wilmington, Delaware, says that some tumours protect themselves from the immune system using another checkpoint, BTLA (an enzyme that was first discovered in a search for ways to protect a fetus from immune rejection). Incyte is testing epcadostat, an existing drug known to inhibit BTLA, as a cancer treatment both alone and in combination with PD-1 blockers.

There are well over 1,000 clinical trials of checkpoint inhibitors going on; what was at first a trickle, then a current, is now a torrent. Some worry that things have gone too far too fast. Jeff Bluestone, who runs the new Parker Institute for Cancer Immunotherapy in San Francisco, says "many of [these trials] are based on minimal data and very limited clinical evidence about what combination will work". Some fear there are too few patients to allow these trials to be run, others that there is too little thought and planning and a lot of duplication of effort. Dr Freeman at Harvard says he has been told there are over 80 Chinese groups developing different PD-1 antibodies.

This enthusiasm may lead to wasted efforts, and even delay progress. But there is no doubt that immunotherapy will from here on be a key part of treatment for a growing number of cancers. Perhaps the most telling measure of its success is that some oncologists have started to complain, quietly, of a shortage of specialist doctors. Patients keep coming back instead of dying.

Building on success

The future, and how to get there

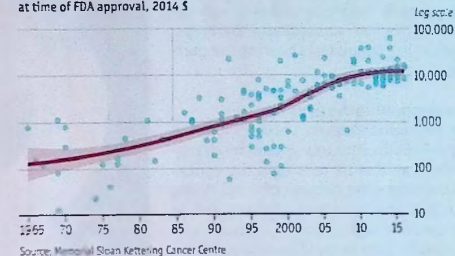
There is a lot more for immunotherapy to do

IMMUNOTHERAPY offers huge promise, both as an addition to established therapies and as a foundation for future ones. Hundreds of trials are pairing CTLA-4, PD-1 or PD-L1 inhibitors with chemotherapy, radiation and targeted therapies. One hope is that the older treatments will increase the range of antigens that the cancer offers for the immune system to latch on to, both by driving further mutations and by killing cancer cells. Dead cells release more antigens.

Then there is the development of further immunotherapies, which is being pursued both by building on the successes of the first checkpoint inhibitors and by using entirely new technologies, such as genome editing. Dr Wolchok at Memorial Sloan Kettering is working on the next generation of immune-modulators. These include new inhibitory compounds for BTLA and TIM-3, another checkpoint. Some researchers are trying to remove further brakes on the system by killing or silencing some of its regulatory cells. Others are looking at molecules which activate the immune system in a similar way to IL-2. Nektar Therapeutics, a biotech firm based in San Francisco, is developing an engineered therapy which does this in a way that should, in principle, encourage tu-

Costly medicine

United States, median monthly cost of cancer drugs at time of FDA approval, 2014 \$



mour-killing T-cells. It is being tested as a combination treatment with an anti-PD-1 drug in five tumour types, including bladder cancer and a hard-to-treat form of breast cancer.

Other approaches seek to make sure that the immune system responds to as many cancer antigens as possible. Viruses genetically engineered to attack cancer cells might be used to this end. Even if such viruses did not kill enough cells to do the cancer much damage, the way in which they kill the cells would release otherwise hard-to-detect antigens that might help the immune system target the tumour better.

Alternatively, the antigens could be provided from outside. Now that immunotherapies have wind in their sails, various old ideas are coming back into vogue. One of them is vaccination. The vaccines with which people are familiar are those against infectious disease. They work by priming the immune system to respond to an antigen associated with a specific pathogen, so that when the system encounters the infection for real it already knows how to fight it. Because some infections can lead to cancer, some of these vaccinations can prevent it. Sometimes, as in vaccination against hepatitis B, which can cause liver cancer, this is an added bonus. Sometimes, as in vaccination against human papilloma virus, which can cause cervical cancer, it is the main point.

But there may be another way to use vaccines against cancer. Equipped with the right antigen, a vaccine might encourage an immune response to a tumour which is already present, but which the immune system has failed to get to grips with. It is an approach that has been frequently tried in the past, and has repeatedly failed. But the availability of checkpoint inhibitors and the ability to pick out the most promising antigens may allow this form of targeting to come into its own.

Neon Therapeutics, Gritstone Oncology, Genocoe Biosciences and other biotech firms are all pursuing the creation of personalised vaccines based on the mutations in an individual tumour. The trick is to find which of the novel antigens its genome says the tumour might be churning out are the most likely to provoke a strong response when served up to the immune system in the form of a vaccine. Jill O'Donnell-Tormey at the Cancer Research Institute (CRI), a non-profit in New York that concentrates on immunotherapies, says that everyone has their favourite algorithm to predict which antigens will get the best response. Together with the Parker Institute in San Francisco, CRI is creating a "bake off" where these algorithms will be tested against each other.

If vaccines work in late-stage cancer—which is where most therapies are tried



Prices are set by what the market will bear

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TECHNOLOGY QUARTERLY | Treating cancer

first—there might be scope for bringing them in sooner, at least in some cancers. In decades to come it is possible to imagine an approach where a tailored vaccine might be the first—and, ideally, the only—response to a blood test showing the presence of a cancer.

Reprogramming the genome

Like immunotherapies, vaccines offer a way to hack the immune system by changing the way that its cells fight the cancer and increasing the number of them doing so. A less circuitous way of doing this is now on offer: reprogram the immune system directly. Take some of its cells out of the body, manipulate them so that they do what you want, encourage them to divide and multiply, then put them back and let them get on with the job.

The technology along these lines that has got furthest is called CAR-T, where CAR stands for "Chimeric antigen receptor". These CARs are produced by splicing together the gene for an antibody that recognises a tumour antigen and the gene for a receptor that sits on the surface of the T-cells; put this new gene into a T-cell and it will be precisely targeted at the tumour. The small clinical trials undertaken to date suggest that this could be extremely effective. A trial of 31 patients with acute lymphoblastic leukaemia brought a complete, and unprecedented, remission in 93% of cases. A CAR-T therapy called Kymriah (tisagenlecleucel), made by the Swiss firm Novartis to treat B-cell acute lymphoblastic leukaemia, was approved for use in America on August 30th.

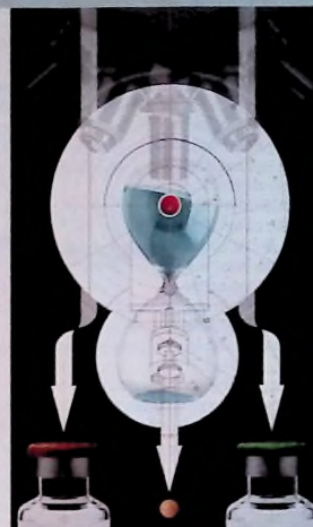
There are two main limitations to CAR-T. One is that so far the T-cells have been programmed to target a molecule, CD19, which is only common to the surface of a few blood cancers. The other is that CAR-T has been known to trigger immune reactions which can prove fatal. Neither problem is obviously insoluble. Editing genes has been made much easier by a new technology known as CRISPR-Cas9, which has already been used to improve the way that CAR-T cells are engineered in mice. It may well eventually allow the receptors used in such therapies to be personalised to the specifics of the patient's cancer. And more precision, as well as experience, should lead to immune responses less likely to run away with themselves.

What such advances will not do, though, is make such treatments cheaper. Novartis's new therapy costs \$475,000. Genome-editing treatments seem likely to be the most expensive cancer treatments the world has yet seen. And that is saying quite a lot, since many of the newer cancer treatments are eye-wateringly pricey (see chart on previous page).

There are various reasons for this. More sophisticated R&D costs a lot. And antibodies are much more expensive to make than the smaller molecules used in older therapies. Generic versions of them are still few and far between. A company that can make antibodies which pass regulatory muster is much better advised to make ones it can sell for a premium.

But the overwhelming factor is that in America, the world's largest market for drugs, prices are set by what the market will bear. When life-saving drugs are available from only one or two providers high prices are a given. This is why pharma companies have piled into oncology over the past decade. They see a market which, by 2025, is forecast to be worth \$45bn-100bn a year.

Not all progress is expensive. Effective early diagnostics could save both money and suffering. The knowledge gained from blood biopsies should allow doctors to tailor treatments better, and avoid drugs that will not work on a given patient. And in a dif-



ferent economic setting bespoke vaccines, gene-editing treatments and the like could in times to come short-circuit rising prices. Molecules made inside the body by reprogrammed cells should be cheaper than those made in expensive cultures. Cutting and splicing the genome could be a great deal cheaper than using scalpels and lasers on the body.

But in the world as it is new cancer therapies will continue to be among the most expensive interventions medicine has to offer, creating a challenge for health systems around the world. And some will disappoint. The immune system's complexity means that it will not always react as doctors hope. Some treatments will prove less effective than at first they seemed. This is a particular problem for cancer drugs, which tend to be approved after comparatively small trials. A recent study of 36 drugs approved between 2008 and 2012 found that 18 did not help patients to live longer. The price of these drugs ranged from \$20,000 to almost \$170,000 per patient.

The incidence of cancer will continue to be dominated by demographics. In developed countries, new therapies may not reduce the chances of getting cancer for some time, simply because older people get more cancers. But the chances of surviving your first cancer, or your next cancer, will improve—and for those with more amenable cancers, and access to the best treatment, they may do so quite quickly. Ever more people will still be told, "I'm afraid you have cancer." But the words will become less fateful, the diagnosis ever less feared.

The cost of progress

When Ms Milley was diagnosed with advanced lung cancer, she went on to Google and read the words "death sentence". It is, alas, fairly typical for patients with terminal cancer to have little idea about their prognosis unless they seek it out. Many might be better served by more openness.

But prognosis is not destiny. Ms Milley started taking Keytruda in December 2015. After two months of treatment her lesions had almost entirely vanished. So far, they have mounted no comeback, and she continues to feel well. She finds the response "amazing".

On any given drug, in any given trial, most people will not be as fortunate. But one of the strange consolations of the current progress being made against cancer is that modern biomedicine makes it possible to learn more from failure than ever before. Huge amounts of the knowledge now saving lives was gained from dead and dying patients, loved ones and friends who lost their fight for life but left a legacy of data. In any given case, that is scant recompense. Put those contributions together, though, and they make a remarkable memorial. ■

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Higher education

University challenge

Which universities do most to boost graduate salaries?

THE Conservative-Lib Dem coalition formed in 2010 wanted universities to expand. Much to the chagrin of thousands of youngsters, it got its way in 2012 by nearly trebling the tuition-fee ceiling to £9,000 (\$14,000) a year. With students shouldering the bulk of the cost of teaching at universities, the government was able to remove the cap on the numbers that universities were allowed to accept. An additional benefit of the shift, hoped officials, was that increased competition for students should drive up standards.

So far competition has not had that desired effect. Most universities charge the maximum, and there has been little change in the quality of teaching. In a bid to ensure that students and taxpayers get better value for money, the government has created a ranking, the "Teaching Excellence Framework" (TEF), which tries to categorise universities by their teaching quality, not their reputation. It hopes to be able to link the fees that universities may charge to instruction.

One way to measure a university's impact is to look at earnings data, which the government also hopes to include in future versions of the TEF. On June 13th the Department for Education released the latest wave of its "longitudinal education outcomes" data, which analyse tax returns to show how much people earn five years after they graduate. To find out which factors are most relevant, *The Economist* has

analysed the data and created a ranking that compares graduates' wages with how much they would have been expected to earn regardless of their university.

Our estimate of expected earnings is built from a statistical model that predicts wages based on the subjects people study, their exam results at school, age, family income, whether they went to private or state school and where the university was located. The difference between the predicted and actual amounts that students earn ought to reflect a university's impact on graduate wages. Our analysis measures how well universities perform compared with the average institution, and is blind to their prestige.

Wait, what about Oxbridge?

The rankings have their flaws. They are based on a single cohort of graduates, who left university in 2009 in the middle of a financial crisis. Using median earnings as a measure understates how much variability there is in graduates' incomes, especially at the top. Nevertheless, the data offer a glimpse of which universities do most to boost earnings. Those at the top are a mix of the illustrious (Nottingham and Oxford) and the unfamiliar (Brunel and Robert Gordon)—see table on next page.

Most of the differences in median earnings can be explained by just two factors: how selective a university is and what subjects their students choose to study there.

Once these are accounted for, graduates' wages are remarkably predictable. Differences in entry tariffs, as defined by UCAS points mostly earned in exams taken at 18, account for nearly 70% of the variation in median earnings. Swots from Cambridge, the most selective university in Britain, earn almost £40,000 a year on average five years after graduating. Their peers at Bedfordshire, the country's least-selective institution, make only half as much.

Subjects which include some element of maths are well-rewarded. Our analysis finds that the five fields with the highest salaries are medicine, veterinary science, economics, engineering and mathematics. By contrast, creative arts, agriculture and communications graduates earn the least. There is a big difference between top earners and poorer ones. After half a decade, medicine and dentistry graduates earn £47,000 a year on average; creative-arts graduates just £20,000.

Encouraging more students to sign up for maths-related degrees may be hard. One problem is that schools in Britain produce few maths whizzes compared with those in other countries, says Anna Vignoles, an economist at Cambridge. Portsmouth, which tops our rankings, provides remedial maths and literacy catch-ups for those needing them. Another difficulty is that universities are not allowed to vary their prices by subject, which means they have little incentive to nudge students towards courses like engineering or science, since it is cheaper to teach the humanities.

Alison Wolf, an economist at King's College London, worries that today's financing system means the number of low-quality courses will grow. A recent study by the Institute for Fiscal Studies, a think-tank, found that funding per student for humanities and business courses has increased by 47% since the reforms of 2012, >>

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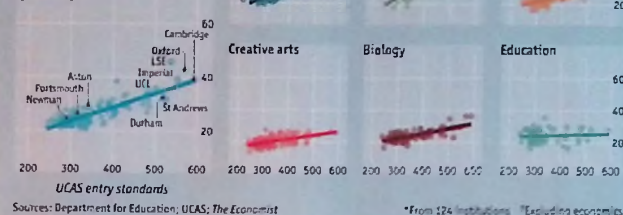
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Hire education

Britain, median salary five years after graduation*, by programme of study 2014, £'000

All subjects
By university



Sources: Department for Education; UCAS; The Economist

*From 124 institutions. †Excluding economics

whereas for laboratory sciences it has risen by just 19%. Yet although some business graduates earn a fortune, there is a large gap between those from higher- and lower-ranked universities. For instance, graduates who study economics and management at Oxford, the most lucrative course according to our data, can expect to be earning three times as much as their contemporaries at the least-selective universities five years after they leave.

Although graduates from research-intensive universities tend to earn more, research quality is no guarantee of a good performance in our rankings. Some of Britain's grandest institutions do well. Oxford, which comes tenth, adds £1,900 to graduate earnings compared with the average university. But the top three universities—Portsmouth, Aston and Newman—are not often found leading the rankings.

Less reputable universities may struggle to attract the brightest students, but they can do plenty to ensure that their graduates do well in the labour market. Many of the universities at the top of our rankings convert bad grades into good jobs. At Newman, a former teacher-training college on the outskirts of Birmingham, classes are small (the staff:student ratio is 16:1), students are few (around 3,000) and all have to do a work placement as part of their degree. (Newman became a university only in 2013, though it previously had the power to award degrees.)

Part of Newman's excellent performance can be explained because more than half its students take education-related degrees, meaning many will work in the public sector. That is a good place for those with bad school grades. Indeed, in courses like education or nursing there is no correlation between earnings and the school grades a university expects (see chart).

Other universities punch above their weight by establishing links with successful industries. At Southampton Solent, on England's south coast, many students take degrees in courses related to the maritime industry. The university, which comes 12th

in our rankings, has one of only five ship-handling lakes in the world, where students can be trained using large-scale models of ships and yachts in a variety of conditions. Similarly, Bournemouth, which comes fourth, has strong links to the film industry and a global reputation for visual effects. John Fletcher, a senior administrator at the university, boasts: "We've won many Oscars."

Some things are beyond a university's control. Graduates from universities in richer areas will tend to earn more—we estimate that, all else being equal, those from a typical university in Glasgow can expect

Great expectations

Britain, median salary five years after graduation 2014, £'000

Highest-ranked universities	Graduate earnings actual	Performance expected*	over/under
Portsmouth	26.2	23.1	3.1
Aston	29.1	26.1	3.0
Newman†	24.4	21.6	2.8
Bournemouth	26.7	23.9	2.7
Robert Gordon	28.9	26.2	2.7
Nottingham	33.0	30.7	2.3
City University	31.1	28.9	2.2
Harper Adams†	28.4	26.2	2.1
Brunel	28.6	26.5	2.0
Oxford	42.8	40.9	1.9
Lowest			
Uni. Campus Suffolk†	20.9	23.3	2.4
Lincoln	21.0	23.5	2.4
Norwich Uni. of the Arts†	17.3	19.9	2.6
Buckingham	23.0	25.7	2.7
Bradford	20.9	23.6	2.7
University of S. Wales	20.8	23.9	3.0
Cumbria	21.7	25.1	3.3
SOAS	27.4	30.9	3.5
Royal Agricultural	27.6	32.2	4.6
St Andrews	31.7	36.6	4.9

Sources: Department for Education; UCAS; DNS; Google Maps; The Economist

*Based on calculations by The Economist. †Recently granted university status

Interactive: See the full rankings and methodology at Economist.com/UniRankings17

to earn £4,200 a year less than graduates of a university in London. Our model attempts to account for geographical differences by being more generous to universities in poor parts of the country. That graduates from Durham and St Andrews, two highly selective universities far from London, fare so poorly is surprising given these adjustments.

First-class universities

The reforms of 2012 increased funding for all universities, but may also have encouraged wasteful spending. Universities now have an incentive to invest in things that will lure more students. There has been a big rise in new buildings. In 2013 universities spent £2.4bn on construction, 43% more than a year earlier, and they have continued to spend at that level since. Spending on marketing has grown (Hull University has the naming rights to the local football team's training ground, for instance). Surveys suggest that students are more satisfied with the state of their campuses, and some were shabby before the investment. But critics question whether this was the best use of extra cash.

Many universities at the top of our rankings are struggling to recruit enough students. This is partly because of the tough climate: there is a demographic dip in the number of 18-year-olds, Brexit is reducing the numbers of European Union students and the abolition of nursing bursaries has had predictable consequences. Yet it also reflects the fact that many students choose their university on the basis of prestige, not teaching quality. Much may depend on whether the government can begin to shift that calculation.

Even if it does, the importance of selectivity in determining graduate outcomes suggests that much of a university education is about "signalling", rather than learning useful skills. This is no bad thing if it makes it easier for employers to spot the best employees, making the labour market more efficient. But, since there has been little improvement in teaching quality, it is not clear how much students have benefited from the increased fees.

Two years ago we built an American ranking using a similar methodology. In comparing the two countries, we find that the school grades of 18-year-olds are a closer predictor of future earnings in Britain than in America. This helps students, because earnings data can provide guidance about what to study, and where. It also helps policymakers, because the analysis shows that it is not always the famous universities that make the biggest difference. Focusing too much attention on elite universities may be ill-advised if much of their success is attributable to the calibre of students they attract. It can be better to study what goes on in Portsmouth and Aston than in Oxford and Cambridge. ■

to earn £4,200 a year less than graduates of a university in London. Our model attempts to account for geographical differences by being more generous to universities in poor parts of the country. That graduates from Durham and St Andrews, two highly selective universities far from London, fare so poorly is surprising given these adjustments.

First-class universities

The reforms of 2012 increased funding for all universities, but may also have encouraged wasteful spending. Universities now have an incentive to invest in things that will lure more students. There has been a big rise in new buildings. In 2013 universities spent £2.4bn on construction, 43% more than a year earlier, and they have continued to spend at that level since. Spending on marketing has grown (Hull University has the naming rights to the local football team's training ground, for instance). Surveys suggest that students are more satisfied with the state of their campuses, and some were shabby before the investment. But critics question whether this was the best use of extra cash.

Many universities at the top of our rankings are struggling to recruit enough students. This is partly because of the tough climate: there is a demographic dip in the number of 18-year-olds, Brexit is reducing the numbers of European Union students and the abolition of nursing bursaries has had predictable consequences. Yet it also reflects the fact that many students choose their university on the basis of prestige, not teaching quality. Much may depend on whether the government can begin to shift that calculation.

Even if it does, the importance of selectivity in determining graduate outcomes suggests that much of a university education is about "signalling", rather than learning useful skills. This is no bad thing if it makes it easier for employers to spot the best employees, making the labour market more efficient. But, since there has been little improvement in teaching quality, it is not clear how much students have benefited from the increased fees.

Two years ago we built an American ranking using a similar methodology. In comparing the two countries, we find that the school grades of 18-year-olds are a closer predictor of future earnings in Britain than in America. This helps students, because earnings data can provide guidance about what to study, and where. It also helps policymakers, because the analysis shows that it is not always the famous universities that make the biggest difference. Focusing too much attention on elite universities may be ill-advised if much of their success is attributable to the calibre of students they attract. It can be better to study what goes on in Portsmouth and Aston than in Oxford and Cambridge.

after graduation	Performance
2015	2015
2014	2014
2013	2013
2012	2012
2011	2011
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Science and technology



Oncology

And then there were five

CHICAGO
Doctors are trying—with some success—to recruit the immune system to help with the war on cancer

THERE are, broadly speaking, four ways to fight cancer. You can cut a tumour out, with surgery. Or you can try one of three different ways of killing it. Radiotherapy targets tumours with radiation. Chemotherapy uses chemicals that poison all rapidly dividing cells, cancerous ones included. "Targeted therapies", as their name suggests, recognise particular features specific to cancer cells.

Singly and in combination, these four types of treatment have contributed to a steady increase in the survival rates for most kinds of cancer. Now they may be joined by a fifth. At this year's meeting of the American Society of Clinical Oncology (ASCO), in Chicago, the assembled researchers heard about the latest progress in "immuno-oncology".

Modern medicine provides every reason to think that the immune system—which, after all, is there to keep the rest of the body safe—can and does attack cancers. People whose immune systems have been weakened, either by disease or by medicines designed to help them tolerate organ transplants, run a greater risk of malignancies. Many risk factors for cancer, such as a bad diet, heavy drinking, stress and smoking are known also to affect the immune system. Exercise, thanks to the boost it gives the body's defences, can improve cancer survival rates.

But attempts to give more specific jolts have been unimpressive. Vaccines have had mixed results. The successful ones,

such as the vaccine for cervical cancer, work by fending off ordinary bugs that happen to be associated with tumour formation. A true cancer vaccine—which would stimulate the immune system to recognise telltale proteins produced by cancer cells themselves—has proved elusive.

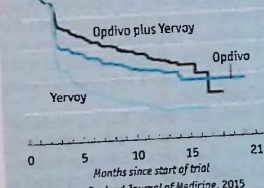
Now, though, a new generation of treatments offers new possibilities. Like targeted treatments, these new approaches often use antibodies—proteins that match up to other proteins with great specificity. Unlike the targeted therapies, though, the new treatments do not directly attack cancerous cells, but instead unleash the immune system on them.

Cancer seems to use three strategies to evade the body's defences, says Edward Bradley of MedImmune, a drug-develop-

Better, longer

Patients whose melanoma did not worsen, %

When treated with:



Source: New England Journal of Medicine, 2015

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73 Portable robots

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Economist.com/science

ment firm based in Maryland. One is to present itself to the body in such a way that the immune system fails to recognise it as something that should be killed. Another is to interfere with the abilities of T-cells, whose duty it is to carry out such killings and which, by hanging around for decades in the body, provide durable immunity to a given disease. Lastly, there are all sorts of ways in which the immune system as a whole can be suppressed.

Oh no you don't

It is the second escape route that many of the firms attending the ASCO meeting hope to cut off. A lot of tumour cells express proteins on their surfaces—so-called "checkpoint" proteins—that bind to complementary molecules on T-cells in a way that persuades the immune system to leave the tumour alone. In 2011 Bristol-Myers Squibb, a drug company, introduced a product called Yervoy, an antibody that binds to one of these deceptive checkpoint proteins, CTLA-4, and thus thwarts its attempts to fool the immune system. Yervoy was the first drug to prolong the lives of patients with melanoma; compared with the standard treatment it reduced their risk of death by about a third. Excitingly, tumours in a small number of patients went away and did not come back.

Last year Merck, another big drugs firm, brought out Keytruda, an antibody that targets a checkpoint protein called PD-1 in a similar way. After a year, 74% of those who received Keytruda were still alive, compared with 58% for those on Yervoy. Bristol-Myers Squibb has since developed Opdivo, another PD-1 drug; Roche and AstraZeneca, two more drug companies, have similar treatments in the pipeline.

Since there are now treatments aimed at more than one checkpoint protein, one question was whether a combination might be more effective than any one drug

alone. Research presented to the conference suggests that it is. Progression-free survival in melanoma (in other words, the amount of time during which a patient's cancer did not become worse) was 6.9 months for those on Opdivo alone and 2.9 months for those taking only Yervoy. Patients given both, though, posted an average time of 11.5 months (see chart).

Checkpoint inhibitors seem to work with other types of cancer, too. Keytruda is effective against some colon cancers and in metastatic head-and-neck cancers. Opdivo, now approved to treat a common form of lung cancer, has shown early promise against liver cancer. Propitious results have also been seen in bladder and kidney cancers. For late-stage lung cancer, where the best treatment is chemotherapy, the fraction of people still alive after five years is typically just 5%. Combinations of immuno-oncology drugs have boosted this to between 20% and 30%. A blizzard of treatment-comparison studies is under way.

Miracles are rare in medicine, and doubly so in oncology. Those encouraging—even spectacular—lung-cancer numbers hide big disparities. Although some patients get a long-term reprieve, there are more for whom checkpoint inhibitors make little difference, or even none at all.

One theory is that some tumours may be employing Dr Bradley's first avenue of escape; instead of (or as well as) trying to hide from a T-cell response, they try to avoid provoking such a response in the first place. Juno Therapeutics, in Seattle, hopes its CAR-T therapy will close that route off, too. This involves extracting T-cells from a cancer patient and modifying them with gene therapy so that they produce a tumour-recognising protein on their surfaces. These cells are multiplied in a dish and put back into the patient's body.

Immune Design, another Seattle firm, wants to do something similar, but from within. It has created a virus that can alter the genetic make-up of dendritic cells, the immunological sergeant-majors responsible for educating T-cells about new threats. Alongside the virus are an antigen and an adjuvant, harmless substances that stimulate T-cell production. The hope is the cocktail will produce an army of primed T-cells.

Checkpoint, counterpoint

Some, maybe many, of the patients who do not respond to checkpoint blockers at the moment might do better if they were given a simultaneous dose of T-cells that already recognised the enemy. It might also be prudent to revisit cancer vaccines to see if their effectiveness improves when used in combination with checkpoint inhibitors. Perhaps they were doing a good job in setting up an immune response only to have the tumours promptly dampen it.

If combining the different forms of immunotherapy with each other offers pro-

mise, so does combining them with the mainstays of cancer treatment—an area in which there is now a lot of research. They might be useful just after surgery, for instance, to mop up any stray cells that the surgeon's knife has missed. They may work well, too, with the targeted therapies that try to choke off tumour growth at the source. Tumours often develop resistance to such targeted therapies; hitting them with a biological double-whammy might make that less common.

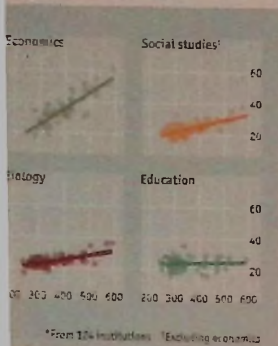
The new treatments look promising. But they do not look cheap. Yervoy costs \$130,000 per patient per year, Opdivo about \$50,000. As more drug companies pile in, patients can hope that competing drugs will hit the market soon. But as the rich world grows older, fatter and therefore more cancer-prone, one salient question is how lucrative immuno-oncology might prove for drugs firms—and how affordable for the insurers and governments that would have to buy them. ■

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MARY A. RUSSELL AND

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CANCER
Vol. 11, No. 2, pp. 1-10
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The Economist August 12th 2017



rankings, has one of only five ship-
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can be trained using large-scale mod-
ships and yachts in a variety of con-
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the university, boasts: "We've won
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areas will tend to earn more—we es-
that, all else being equal, those from
al university in Glasgow can expect

it expectations

Institution	Median salary five years after graduation (£000)		Performance ratio
	Graduate earnings	Performance ratio	
2010-11	actual	expected	under
South	26.2	23.1	2.1
1	29.1	26.1	3.0
2	24.4	21.6	2.8
South	26.7	23.9	2.7
Gordon	28.9	26.2	2.7
ham	33.0	30.7	2.2
University	31.1	28.9	2.2
Adams	28.4	26.2	2.1
	28.6	26.5	2.0
	42.8	40.9	1.9

St. Safford	20.9	23.3	2.4
	21.0	23.5	2.4
Uni. of the Arts	17.3	19.9	2.6
am	23.0	25.7	2.7
	20.9	23.6	2.7
y of S. Wales	20.8	23.9	3.0
	21.7	25.1	3.3
	27.4	30.9	3.5
cultural	27.6	32.2	4.6
	21.7	36.6	4.9

*Based on calculations by The Economist. Recently granted university status.

See the full rankings and methodology at www.economist.com/UniRankings27

to earn £4,200 a year less than graduates of a university in London. Our model attempts to account for geographical differences by being more generous to universities in poor parts of the country. That graduates from Durham and St Andrews, two highly selective universities far from London, fare so poorly is surprising given these adjustments.

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Helicopter technology

Chop-chop

Strange new rotor-blade arrangements make for faster helicopters

HELICOPTERS have clear advantages over fixed-wing aircraft. But they also have a big drawback: they are slow. The fastest struggle to exceed about 320kph (200mph)—less than the cruising speed of a second-world-war-era propeller plane.

At an aircraft-testing facility in Florida, though, a strange-looking helicopter has taken to the skies that may help to close that gap. There are two striking things about the Sikorsky S-97 Raider. The first is its set of two rotors, mounted one on top of the other and turning in opposite directions around a central shaft—much like the radio-controlled helicopters sold in toy shops. The second is the absence of a tail rotor. In its place sits a backwards-facing "pusher" propeller.

Start with the rotors. Such a "coaxial" arrangement has been found in a few helicopters over the years. Kamov, a Russian company, makes one that is often used to lift heavy loads. But the mechanical com-

The new treatments look promising. But they do not look cheap. Yervoy costs \$130,000 per patient per year, Opdivo about \$150,000. As more drug companies pile in, patients can hope that competing drugs will hit the market soon. But as the rich world grows older, fatter and therefore more cancer-prone, one salient question is how lucrative immuno-oncology might prove for drugs firms—and how affordable for the insurers and governments that would have to buy them. ■

plexity and the risk of the blades hitting one another has made such machines unpopular. Modern engineering methods, together with the use of stiffer carbon-composite blades that are less likely to flex and collide, promise to reduce such problems.

And twin rotors provide several advantages. They offer inherent stability—which is why children can fly the model choppers equipped with them. The single set of rotors on a standard helicopter produces a rotational force, or torque. Without a tail rotor to counter it, the fuselage would spin in the opposite direction to the rotors. The counter-rotating blades of the Sikorsky machine cancel each other's torque.

They also allow more speed. Like a fixed wing, a rotor blade provides lift proportional to the amount of air flowing over it. In forward flight an advancing blade moves faster, relative to the surrounding air, than a retreating one. That creates uneven lift that tries to roll the chopper over. ■

The Economist June 6th 2015

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Coaxial rotor-blade arrangements make for faster helicopters

have clear advantages over fixed-wing aircraft. But they also have drawbacks: they are slow. The fastest helicopter can exceed about 320kph, less than the cruising speed of a modern propeller plane. The testing facility in Florida, the Sikorsky S-97 Raider, has a coaxial rotor helicopter that may help to close the gap. There are two striking things about the S-97. The first is its rotors, mounted one on top of the other, turning in opposite directions on a common central shaft—much like the helicopters sold in toy shops. The second is the absence of a tail rotor. The S-97 sits a backwards-facing tail rotor. Such a “coaxial” rotor has been found in a few helicopters in the past few years. Kamov, a Russian helicopter designer, is one that is often used to build them. But the mechanical com-

plexity and the risk of the blades hitting one another has made such machines unpopular. Modern engineering methods, together with the use of stiffer carbon-composite blades that are less likely to flex and collide, promise to reduce such problems.

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RELATION OF MITOTIC ACTIVITY TO THE EFFECTS OF X-RAYS AND NITROGEN MUSTARD AS INDICATED BY THE GROWTH OF CORN SEEDLINGS

MARY A. RUSSELL AND FRANCIS J. MICHELINI

Reprinted for private circulation from
CANCER RESEARCH
Vol. 11, No. 9, pp. 687-93, September 1951
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PRINTED IN U.S.A.

Relation of Mitotic Activity to the Effects of X-Rays and Nitrogen Mustard as Indicated by the Growth of Corn Seedlings*

MARY A. RUSSELL AND FRANCIS J. MICHELINI†

(Department of Biological Sciences, University of Delaware, Newark, Del.)

In their review of the early research on mustard compounds, Gilman and Philips (3) list several striking similarities between the biological effects of these chemicals and radiation. They report that "Actively proliferating cells are selectively vulnerable to the cytotoxic action of the mustards." There is general agreement that, in most cases, dividing cells are more sensitive to radiation than resting cells, but recent literature indicates a conflict of opinion as to whether dividing cells are necessarily more sensitive to the effects of mustards. Friedenwald and Buschke (2) have discussed the difficulty of the problem. They point out that while some tissues with many dividing cells, such as bone marrow and intestinal mucosa, are very sensitive to mustard, a tissue like liver, with active cell division stimulated by partial hepatectomy, is comparatively insensitive.

The purpose of this investigation is to compare the sensitivity of tissue containing many dividing cells with the *same kind of tissue* in which little or no cell division is present at the time of exposure to either x-rays or nitrogen mustard. The root of the corn seedling has been found to be a satisfactory organism for studying the effects of both x-rays (5) and nitrogen mustard (6). It is particularly suitable for this problem, because tissue may be obtained with no cell division or with varying percentages of dividing cells by controlling the length of the germination period or by chemical treatment.

MATERIALS AND METHODS

Corn seed of the "U.S. 13" strain was treated for 5 minutes in a 0.05 per cent mercuraphen solution to prevent the growth of mold. After soaking

in water for 1 hour, the seed was allowed to germinate at 30° C. Tall 600-ml. beakers were lined with filter paper, then loosely filled with moist sphagnum moss (buffered to maintain a pH of about 5), covered with petri dish lids, and autoclaved at 15 lb. pressure for 30 minutes. After the desired period of germination, the seedlings were exposed to x-rays or to a solution of methylbis(β -chloroethylamine hydrochloride, designated as HN2. After treatment, about fifteen seedlings were placed in each beaker so that the roots grew down between the paper and the glass. The beakers were incubated at 30° C. A mark was placed on every beaker to indicate the position of the first seedling, so that a record could be kept of each individual. The length of the primary roots was generally measured twice daily, and observations were made at more frequent intervals to determine the time at which the first lateral roots appeared. If the primary roots reached the bottom of the beaker (about 120 mm.), they were classed as survivors, and roots whose length remained constant for 3 days were recorded as having stopped growing.

The x-ray machine used in these experiments was operated at 120 kv. and 10 ma with a 1.5-mm. aluminum filter. The output intensity was 72 r/min. During radiation the seeds were placed with embryo side up and covered with cellophane to prevent dehydration.

The HN2 used in these investigations was furnished by the Army Chemical Center, Maryland. A fresh solution was prepared just before each experiment, with distilled water of about pH 5.7, unless otherwise stated. A temperature of 28°-30° C. was maintained during exposure of the seedlings to HN2, and they were washed for 5 minutes in running tap water to remove all traces of the chemical.

Two methods were used to obtain tissue with little or no cell division for comparison with similar tissue containing a higher percentage of dividing cells.

* These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Delaware (NR 164-858).

† Present address: Botany Department, University of Pennsylvania, Philadelphia, Pa.

Received for publication March 16, 1951.

combining them with the cancer treatment—an area in which a lot of research is now being done. They will just after surgery, for instance, to kill any stray cells that the surgeon has missed. They may also be used with the targeted therapies that are off target at the time of surgery, often develop resistance to the therapies; hitting them at the time of surgery might be a double-whammy might be common.

The new treatments look promising. But they do not look cheap. Yervoy costs \$130,000 per patient per year, Opdivo about \$150,000. As more drug companies pile in, patients can hope that competing drugs will hit the market soon. But as the rich world grows older, fatter and therefore more cancer-prone, one salient question is how lucrative immuno-oncology might prove for drug firms—and how affordable for the insurers and governments that would have to buy them. ■



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top

Coaxial-blade arrangements make for faster helicopters

have clear advantages over conventional aircraft. But they also have disadvantages: they are slow. The fastest conventional helicopter exceeds about 320kph in the cruising speed of a conventional propeller plane. The Sikorsky facility in Florida, for example, is looking for a helicopter that may help to close the gap between the two striking things about the UH-60 Raider. The first is mounted one on top of the other in opposite directions—much like the helicopters sold in toy shops. The second is the absence of a tail rotor.

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METHOD A

Use of embryos germinated for different periods of time.—Stained sections of the radicle tips of embryos germinated for 12 hours showed no mitotic figures, but after 15 or 16 hours of germination, about 15 per cent of the cells in the meristematic region of the tip were in some stage of mitosis. Therefore, 15 or 16 hours were allowed for germination when tissue with cell division was desired, and 11 or 12 hours when the experiment called for tissue without cell division. These two germination periods were chosen because it was desirable to use tissue in as nearly the same physiological condition as possible, but at the same time to make certain that the 4 hours' difference would divide the two groups into one with cell division and one without cell division. In preparing for these experiments, one set of seeds was started germinating 4 hours before the other, so that both sets would be ready for exposure to either x-rays or HN2 at the same time. In the HN2 experiments, controls were placed in distilled water during the time when the seedlings were soaked in the HN2 solution.

METHOD B

Use of seedlings treated with 3,5-dinitro-o-cresol (DNC), to inhibit cell division for comparison with normal seedlings.—Clowes and Krahel (1) reported that DNC was a reversible mitotic inhibitor when its action was tested on sea urchin eggs. Beck and Russell¹ later found that corn roots would resume normal growth after cell division was decreased in the root tip by means of exposure to DNC. Seedlings with roots about 10 mm. long were divided into two groups, as follows: the roots of group 1 were immersed in a 10^{-5} M solution of DNC for 19 hours at 20° C. and washed in running water for 5 minutes; the roots in group 2 were kept in distilled water for the same time at the same temperature. Both groups were then ready for exposure to either radiation or HN2. During the soaking period, the DNC-treated roots showed no appreciable growth, whereas the controls grew normally. Charts 2 and 3 show that the DNC-treated roots quickly recovered, and their growth soon paralleled that of the controls. It was noted in sections prepared from root tips fixed at the end of the 19-hour soaking period that about 22 per cent of the total cells in the meristematic region were dividing in group 1, while in the normal group (2) about 38 per cent were in some stage of division. Part of each group was kept untreated as controls.

¹ L. V. Beck and M. A. Russell, unpublished report, 1934.

RESULTS

Table 1 shows that, in every experiment, the percentage of roots which stopped growing was always higher in the group of seedlings with cell division present at the time of radiation. When the averages from all the experiments are compared, the difference is very striking ($P < 0.001$). A study of the lateral roots showed that the seedlings treated at a time after cell division had started were more sensitive to radiation than the seedlings in which there was no cell division at the time of treatment. In the group in which cell division was present, only 60 per cent of the seedlings produced laterals, while in the second group 97 per cent had laterals. In the first group, the initial appearance of the laterals was delayed longer than in the second group. All these facts indicate that the seedlings in the group with cell division suffered the greater amount of radiation injury.

In Table 2, the behavior of the primary roots after treatment with HN2 shows that there was much less difference in sensitivity to this agent between the group with dividing cells and the group with no cell division at the time of exposure to the HN2 solution. The difference is not significant ($0.01 < P < 0.02$). A study of the lateral roots showed that, although the first of these appeared in both groups at the same time, they were more numerous in the group without cell division, showing these seedlings to be slightly more resistant to HN2.

Chart 1 shows the growth curves of the primary roots which died in two typical experiments using Method A (Table 1, Exp. 3, and Table 2, Exp. 3). The shape of the curve made from data from roots treated with HN2 is very similar to the curve made from the data from the x-rayed roots, and the final length of the primary roots is about the same in both cases.

Chart 2 shows the behavior of the primary roots in a typical experiment (Exp. 3) from Table 3. In each experiment of this series, a larger percentage of roots stopped growing in the normal cell division group than in the reduced cell division group. The difference is highly significant ($P < 0.001$). Again, the laterals appeared later in the former group, and at the end of the experiment only 83 per cent of these seedlings had laterals, as compared to the presence of laterals in 99 per cent of the group with reduced mitotic activity. All this evidence indicates that seedlings with a normal mitotic index were more sensitive to radiation than those in which the number of dividing cells was reduced by DNC treatment.

Chart 3 was constructed on the basis of Exp. 3 in the series recorded in Table 4. This table shows

that when the two groups of seedlings were treated with identical doses of HN2, the normal rate of cell division was more sensitive than the group with reduced cell division by DNC. This difference is significant ($0.01 < P < 0.02$). While all the seedlings at the end of the experiment showed a slight division group showed a slight sensitivity to HN2, as indicated by seedlings appearing somewhat with the reduced cell division group.

DISCUSSION

A comparison of the "cell division" groups in Tables 1 and 2 shows that these doses of radiation or HN2 of roots which stopped growing were the same. The average length of the roots at the time of death and the number of lateral root growth were approximately equal in about the same amount of time.

COMPARISON OF THE EFFECTS OF X-RAY AND HN2 ON YOUNGER EMBRYOS

EXP. NO.	X-RAY DOSE	GERMINATION TIME (hr.)	No. of roots
1	4,000 r	15	2
2	3,500 r	15	2
3	4,000 r	16	2
4	4,000 r	16	2
5	4,000 r	16	2
6	4,000 r	16	1
Total			14

* Weighted averages.

COMPARISON OF THE EFFECTS OF X-RAY AND HN2 ON YOUNGER EMBRYOS

EXP. NO.	HN2 DOSE 5.2×10^{-2} M (min.)	GERMINATION TIME (hr.)	No. of roots
1	30	15	29
2	30	15	36
3	30	16	38
4	30	16	33
5	30	15	31
6*	30	16	18

Total

* pH of water higher, HN2 solution pH 8.6.
† Weighted averages.

METHOD A

embryos germinated for different periods of time. Sections of the radicle tips of embryos germinated for 12 hours showed no mitotic activity after 15 or 16 hours of germination, whereas 100 per cent of the cells in the meristematic region of the tip were in some stage of mitosis. Embryos 15 or 16 hours were allowed for germination when tissue with cell division was demonstrated 11 or 12 hours when the experiment was run. These two periods were chosen because it was possible to use tissue in as nearly the same physiological condition as possible, but at the same time maintain that the 4 hours' difference would not affect the two groups into one with cell division and one without cell division. In preparing for experiments, one set of seeds was started germinating 4 hours before the other, so that both would be ready for exposure to either x-rays or HN2 at the same time. In the HN2 experiments, controls were placed in distilled water during the time when the seedlings were soaked in solution.

METHOD B

Seedlings treated with 3,5-dinitro-o-cresol inhibit cell division for comparison with controls.—Clowes and Krah (1) reported that a reversible mitotic inhibitor when tested on sea urchin eggs. Beck and Russell (2) found that corn roots would resume growth after cell division was decreased in corn roots by means of exposure to DNC. Seedlings about 10 mm. long were divided into groups, as follows: the roots of group 1 were soaked in a 10^{-5} M solution of DNC for 20° C. and washed in running water; the roots in group 2 were kept in distilled water for the same time at the same temperature. Both groups were then ready for either radiation or HN2. During the experiment, the DNC-treated roots showed no growth, whereas the controls grew normally. Parts 2 and 3 show that the DNC-treated roots quickly recovered, and their growth was not affected that of the controls. It was noted that after a soaking period that about 22 per cent of the cells in the meristematic region of the root tips in group 1, while in the normal group 2, 38 per cent were in some stage of mitosis. Of each group was kept untreated

RESULTS

Table 1 shows that, in every experiment, the percentage of roots which stopped growing was always higher in the group of seedlings with cell division present at the time of radiation. When the averages from all the experiments are compared, the difference is very striking ($P < 0.001$). A study of the lateral roots showed that the seedlings treated at a time after cell division had started were more sensitive to radiation than the seedlings in which there was no cell division at the time of treatment. In the group in which cell division was present, only 60 per cent of the seedlings produced laterals, while in the second group 97 per cent had laterals. In the first group, the initial appearance of the laterals was delayed longer than in the second group. All these facts indicate that the seedlings in the group with cell division suffered the greater amount of radiation injury.

In Table 2, the behavior of the primary roots after treatment with HN2 shows that there was much less difference in sensitivity to this agent between the group with dividing cells and the group with no cell division at the time of exposure to the HN2 solution. The difference is not significant ($0.01 < P < 0.02$). A study of the lateral roots showed that, although the first of these appeared in both groups at the same time, they were more numerous in the group without cell division, showing these seedlings to be slightly more resistant to HN2.

Chart 1 shows the growth curves of the primary roots which died in two typical experiments using Method A (Table 1, Exp. 3, and Table 2, Exp. 3). The shape of the curve made from data from roots treated with HN2 is very similar to the curve made from the data from the x-rayed roots, and the final length of the primary roots is about the same in both cases.

Chart 2 shows the behavior of the primary roots in a typical experiment (Exp. 3) from Table 3. In each experiment of this series, a larger percentage of roots stopped growing in the normal cell division group than in the reduced cell division group. The difference is highly significant ($P < 0.001$). Again, the laterals appeared later in the former group, and at the end of the experiment only 83 per cent of these seedlings had laterals, as compared to the 99 per cent of the group with the normal cell division. All this evidence indicates that seedlings with a normal mitotic index were more sensitive to radiation than those in which the number of dividing cells was reduced by DNC treatment.

Chart 3 was constructed on the basis of Exp. 3 in the series recorded in Table 4. This table shows

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that when the two groups of seedlings were treated with identical doses of HN2, the group with a normal rate of cell division was only slightly more sensitive than the group with cell division reduced by DNC. This difference is not significant, since $0.01 < P < 0.02$. While all the roots had laterals at the end of the experiments, the normal cell division group showed a slightly increased sensitivity to HN2, as indicated by the laterals in these seedlings appearing somewhat later than in the group with the reduced cell division.

DISCUSSION

A comparison of the "cell division present" columns in Tables 1 and 2 shows that, after the use of these doses of radiation or HN2, the percentages of roots which stopped growing were nearly the same. The average length of the primary roots at the time of death and the number of seedlings in which lateral root growth was entirely suppressed were approximately equal in both groups. Since about the same amount of injury resulted from

the use of the two agents, the doses of x-rays and HN2 used in these experiments with 16-hour germinated embryos are biologically equivalent to one another. However, if younger embryos containing no dividing cells were used as the test material, with the same dose of HN2, a considerably larger dose of x-rays would be required to kill the same percentage of roots.

It will be noted in Table 1 that 3,500 and 4,000 r were used to stop the growth of 73 per cent of the 16-hour germinated embryos, but the results of Table 3 show that only 1,700 and 1,750 r were required to produce the same mortality in the group with the normal rate of mitosis. The greater sensitivity of the older seedlings as shown in Table 3 may be due, in part, to the increase in hydration and general metabolic rate, but it is probable that most of the increased sensitivity can be attributed to the higher mitotic rate.

One obvious reason for the effectiveness of the more dilute solution of HN2 used to treat the older seedlings (Table 4) is that these roots could ab-

TABLE 1
COMPARISON OF THE EFFECT OF X-RAYS ON CORN EMBRYOS CONTAINING DIVIDING CELLS AND YOUNGER EMBRYOS WITH NO DIVIDING CELLS AT THE TIME OF RADIATION

Exp. No.	X-RAY DOSE	GERMINATION TIME (hr.)	CELL DIVISION PRESENT (15 PER CENT)				CELL DIVISION ABSENT			
			Controls		Treated		Controls		Treated	
			No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing
1	4,000 r	15	31	10	13	30	11	—	—	—
2	3,500 r	15	—	—	14	43	28	4	15	20
3	4,000 r	16	31	0	29	97	12	0	14	21
4	4,000 r	16	32	0	25	84	31	0	30	20
5	4,000 r	16	32	6	29	86	32	0	29	41
6	4,000 r	16	16	0	32	62	33	0	28	15
					16	0	16	0	28	10
Total			142	3.5*	142	73	140	0.7*	144	22*

* Weighted averages.

TABLE 2
COMPARISON OF THE EFFECTS OF HN2 ON CORN EMBRYOS CONTAINING DIVIDING CELLS AND YOUNGER EMBRYOS WITH NO DIVIDING CELLS AT THE TIME OF TREATMENT

Exp. No.	HN2 DOSE 5.2×10^{-5} M (min.)	GERMINATION TIME (hr.)	CELL DIVISION PRESENT (15 PER CENT)				CELL DIVISION ABSENT			
			Controls		Treated		Controls		Treated	
			No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing
1	30	15	29	0	29	45	11	20	10	28
2	30	15	36	0	36	55	11	36	0	33
3	30	16	38	0	33	55	12	35	0	30
4	30	16	33	0	32	62	12	34	0	72
5	30	15	31	0	53	75	11	31	0	41
6*	30	16	18	0	70	97	17	0	69	91
Total			182	0	253	71†	173	1.2†	273	62†

* pH of water higher, HN2 solution pH 6.0.

† Weighted averages.

Combining them with the newer treatment—an area in which a lot of research is being done—just after surgery, for instance, any stray cells that they have missed. They may be able to target therapies off tumour growth at the often develop resistance therapies; hitting them double-whammy might be common.

The new treatments look promising. But they do not look cheap. Yervoy costs \$130,000 per patient per year, Opdivo about \$150,000. As more drug companies pile in, patients can hope that competing drugs will hit the market soon. But as the rich world grows older, fatter and therefore more cancer-prone, one salient question is how lucrative immuno-oncology might prove for drug firms—and how affordable for the insurers and governments that would have to buy them. ■



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Blade arrangements make for faster helicopters

have clear advantages over aircraft. But they also have drawbacks: they are slow. The fastest about 320kph, the cruising speed of a propeller plane. The new facility in Florida, looking like a helicopter, may help to close the gap. Two striking things about the 97 Raider. The first is that it is mounted on top of a fuselage in opposite directions—much like the rotors of a helicopter. The second is that it has a tail rotor in the absence of a tail boom.

Such a “coaxial” arrangement is found in a few helicopters. Kamov, a Russian design, is often used to land on the mechanical com-

plexity and the risk of the blades hitting one another has made such machines unpopular. Modern engineering methods, together with the use of stiffer carbon-composite blades that are less likely to flex and collide, promise to reduce such problems.

And twin rotors provide several advantages. They offer inherent stability—which is why children can fly the model choppers equipped with them. The single set of rotors on a standard helicopter produces a rotational force, or torque. Without a tail rotor to counter it, the fuselage would spin in the opposite direction to the rotors. The counter-rotating blades of the Sikorsky machine cancel each other's torque.

They also allow more speed. Like a fixed wing, a rotor blade provides lift proportional to the amount of air flowing over it. In forward flight an advancing blade moves faster, relative to the surrounding air, than a retreating one. That creates uneven lift that tries to roll the chopper over. ▶

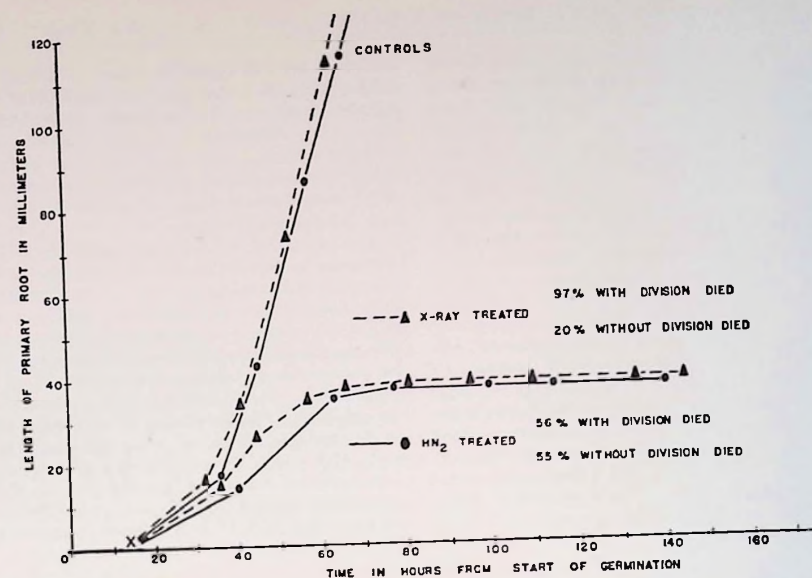


CHART 1.—Growth curves of primary roots of corn seedlings which died after exposure to 4,000 r and 5.2×10^{-6} M HN₂ solution for 30 minutes, respectively.

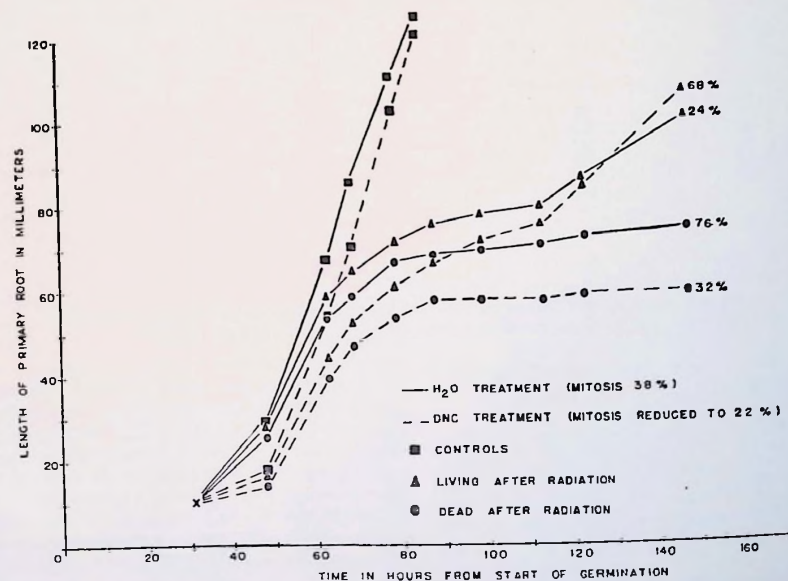


CHART 2.—Growth curves of primary roots of seedlings radiated with 1,700 r after soaking in water (38 per cent mitosis) and after soaking in DNC (22 per cent mitosis).

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sorb the solution freely, and younger embryos (Table 1) with intact pericarp.

The embryos of the two groups differed in the number of dividing cells, the older group having more tissue of the older group which had a greater diameter. The last two variables were eliminated which made use of seedlings.

COMPARISON OF THE EFFECTS OF X-RAY AND ON SEED

EXP. NO.	X-RAY DOSE
1	1,750 r
2	1,700 r
3	1,700 r
4	1,700 r

Total
* Weighted averages.

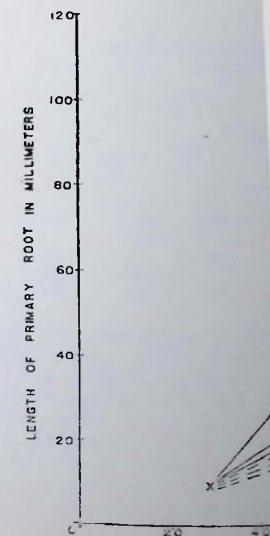


CHART 3.—Growth curves of primary roots of seedlings radiated with 1,700 r after soaking in water (38 per cent mitosis) and after soaking in DNC (22 per cent mitosis).

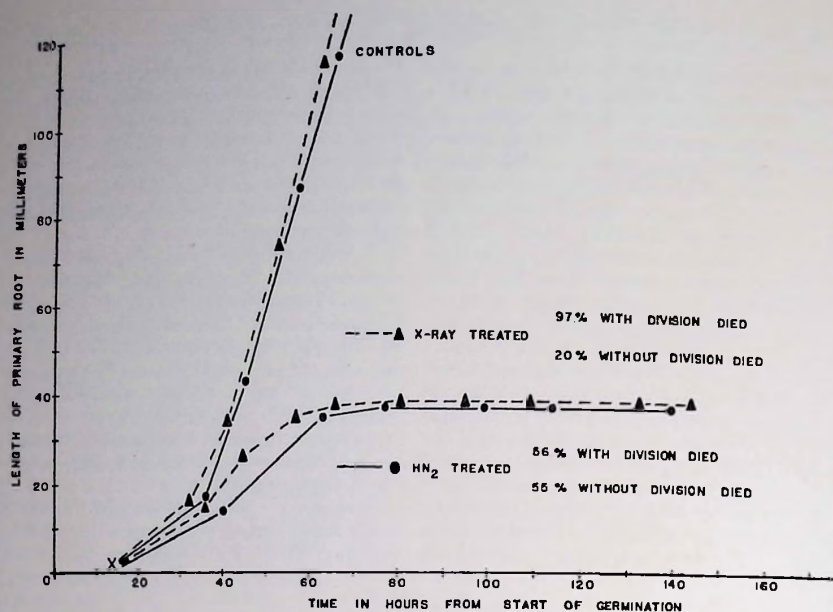


CHART 1.—Growth curves of primary roots of corn seedlings which died after exposure to 4,000 r and 5.2×10^{-4} M HN₂ solution for 30 minutes, respectively.

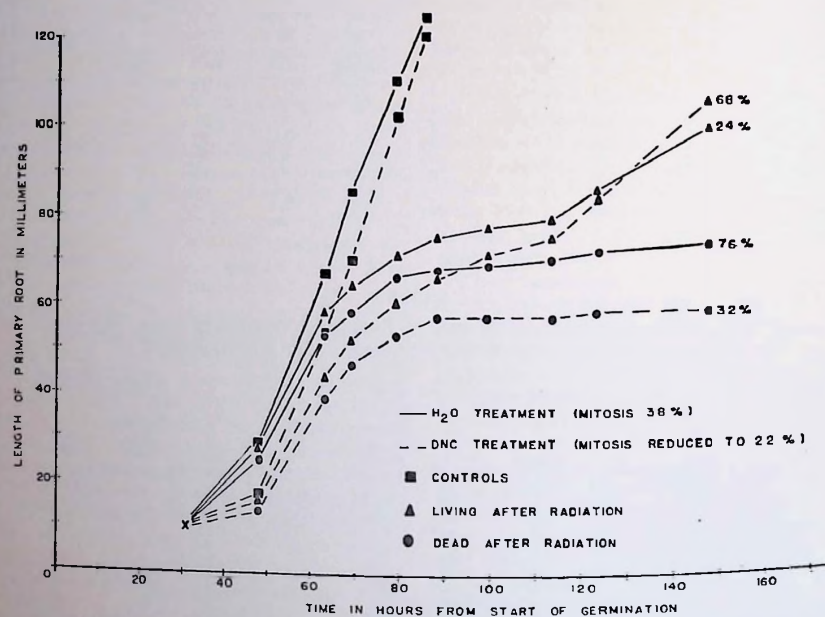


CHART 2.—Growth curves of primary roots of seedlings radiated with 1,700 r after soaking in water (38 per cent mitosis) and after soaking in DNC (22 per cent mitosis).

sorb the solution freely, while the roots of all the younger embryos (Table 2) were protected by the intact pericarp.

The embryos of the two age groups used in Method A differed from one another not only in the number of dividing cells, but also in that the tissue of the older group was more highly differentiated and had a greater degree of hydration. The last two variables were eliminated in Method B, which made use of seedlings of the same age.

When DNC seedlings were radiated, they were less sensitive than normal seedlings. If the respiration rate had been increased by the DNC pretreatment, one would expect sensitivity to increase also. This tendency may have been present and its effect masked by the effect of decreasing rate of mitosis on lowering sensitivity. The possibility of any interaction between DNC and HN₂ would seem to be ruled out by the results of the following experiment: when HN₂ was dissolved in a 10^{-6} M

TABLE 3
COMPARISON OF THE EFFECT OF X-RAYS ON NORMAL CORN SEEDLINGS WITH 38 PER CENT DIVIDING CELLS AND ON SEEDLINGS TREATED WITH DNC TO REDUCE CELL DIVISION TO 22 PER CENT

Exp. No.	X-ray dose	Normal cell division (38 per cent)		Cell division reduced to 22 per cent by DNC	
		Controls	Treated	Controls	Treated
		No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing
1	1,750 r	34	0	26	4
2	1,700 r	16	0	20	0
3	1,700 r	18	0	17	0
4	1,700 r	15	0	16	0
Total		83	0	141	72*
				170	1*
				153	31*

* Weighted averages.

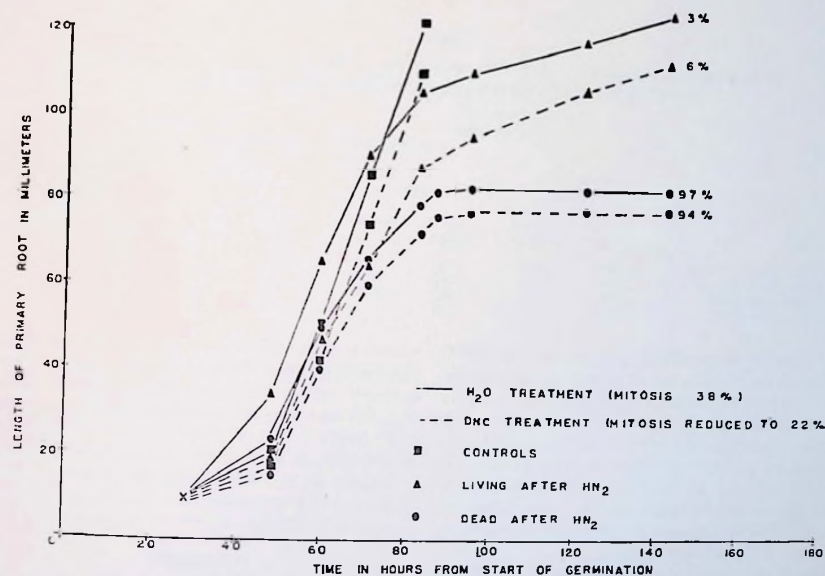


CHART 3.—Growth curves of primary roots of seedlings exposed to 2.6×10^{-4} M HN₂ for 20 minutes after soaking in water (38 per cent mitosis) and after soaking in DNC (22 per cent).

Combining them with the cancer treatment—an area in which a lot of research. They are just after surgery, for instance, to kill any stray cells that the treatment has missed. They may also have the targeted therapies off-tumour growth at the time of surgery, often develop resistance to the therapies; hitting them with a double-whammy might be more effective.

The new treatments look promising. But they do not look cheap. Yervoy costs \$130,000 per patient per year, Opdivo about \$150,000. As more drug companies pile in, patients can hope that competing drugs will hit the market soon. But as the rich world grows older, fatter and therefore more cancer-prone, one salient question is how lucrative immuno-oncology might prove for drug firms—and how affordable for the insurers and governments that would have to buy them. ■



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Coaxial-blade arrangements make for faster helicopters

have clear advantages over a conventional helicopter. But they also have drawbacks: they are slow. The fastest, the X-45, exceeds about 320kph in the cruising speed of a conventional propeller plane.

At a testing facility in Florida, a Sikorsky Black Hawk helicopter has been modified to have two striking things. The first is that it is mounted on a single set of rotors on a standard helicopter produces a rotational force, or torque. Without a tail rotor to counter it, the fuselage would spin in the opposite direction to the rotors. The counter-rotating blades of the Sikorsky machine cancel each other's torque.

They also allow more speed. Like a fixed wing, a rotor blade provides lift proportional to the amount of air flowing over it. In forward flight an advancing blade moves faster, relative to the surrounding air, than a retreating one. That creates uneven lift that tries to roll the chopper over. ▶▶

plexity and the risk of the blades hitting one another has made such machines unpopular. Modern engineering methods, together with the use of stiffer carbon-composite blades that are less likely to flex and collide, promise to reduce such problems.

And twin rotors provide several advantages. They offer inherent stability—which is why children can fly the model choppers equipped with them. The single set of rotors on a standard helicopter produces a rotational force, or torque. Without a tail rotor to counter it, the fuselage would spin in the opposite direction to the rotors. The counter-rotating blades of the Sikorsky machine cancel each other's torque.

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solution of DNC instead of water, seedlings exposed to this solution behaved the same as seedlings treated with the same concentration of HN2 made up in water.

Table 5 summarizes the data presented in the other tables. The mortality figures in line 2 show that, with the use of either method, corn root tissue with the greater amount of cell division present at the time of radiation was much more sensitive to x-rays than the same tissue with less cell division. In line 3 the figures from both methods

of Henshaw and Francis (4), who reported similar results with wheat seedlings. The fact that the roots whose rate of cell division was reduced by DNC were less sensitive to x-rays than normal roots of the same age is also in agreement with the general conception that sensitivity to radiation is usually directly proportional to the mitotic index of the tissue.

The experimental data presented here confirm the statement by Strong (7) that nitrogen mustard seemed to affect cells even before there was any

TABLE 4
COMPARISON OF THE EFFECT OF HN2 ON NORMAL CORN SEEDLINGS AND ON SEEDLINGS TREATED WITH DNC TO REDUCE CELL DIVISION

Exp. No.	HN2 dose $2.6 \times 10^{-4} M$ (min.)	NORMAL CELL DIVISION (58 PER CENT)		CELL DIVISION REDUCED TO 22 PER CENT BY DNC	
		Controls	Treated	Controls	Treated
		No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing
1	16	17	0	17	0
2	18	18	0	17	0
3*	20	19	0	18	0
4	20	33	6	34	0
Total		88	2†	86	0

* pH of water higher, HN2 solution pH 6.6.

† Weighted averages.

TABLE 5
COMPARISON OF THE EFFECTS OF X-RAYS OR HN2 WHEN APPLIED TO TISSUE WITH VARYING AMOUNTS OF CELL DIVISION OBTAINED BY TWO DIFFERENT METHODS

	METHOD A (TABLES 1 AND 2)				METHOD B (TABLES 3 AND 4)			
	15- or 16-hr. germinated embryos		11- or 12-hr. germinated embryos		Normal 48-hr. seedlings		DNC-treated 48-hr. seedlings	
	No. of plants	Per cent mortality	No. of plants	Per cent mortality	No. of plants	Per cent mortality	No. of plants	Per cent mortality
Controls for radiation	142	3.5	140	0.7	83	0	170	1
Treated with X-rays	142	73.0	144	22.0	141	72	153	31
Treated with HN2	253	71.0	273	62.0	229	53	243	46
Controls for HN2	182	0	173	1.2	88	2	86	0

show that, when seedlings were exposed to HN2, the variation in the amount of cell division at the time of exposure made no significant difference in the sensitivity of the roots. These results show that under the conditions of these experiments differences in mitotic activity during exposure affected sensitivity to HN2 to a negligible degree, while the radiosensitivity of the same tissue increased with the amount of cell division present at the time of exposure to x-rays.

The data indicating a marked rise in sensitivity of embryo roots to radiation, at about the time that mitosis commences, agree with the findings

evidence of cell division. It may be that, although the seedlings showed the effects of x-rays and HN2 in many similar ways, the action of the two agents was different when the reaction of resting cells was compared to that of dividing cells. Further experimentation may show whether this difference is mainly in the initial damage or whether the dividing cells are better able to recover after exposure to HN2 than after x-ray treatment.

SUMMARY

1. Two methods were used to obtain corn seedlings with little or no cell division for comparison

with similar tissue containing no cell division:

a) Embryos were germinated in tips containing no cell division with about 15 per cent cells dividing.

b) Older seedlings were treated with 2,2'-bis(4-chlorophenyl)propane, which reduced cell division in the root tip from 22 per cent.

2. When either method was used, seedlings with the higher rate of cell division were slightly more sensitive to bis(β-chloroethyl)amine than the group with the lower rate.

3. When either method was used, seedlings which had the greater amount of cell division at the time of exposure were significantly more than were fewer dividing cells.

4. Under the same experimental conditions, corn root tissue showed a sensitivity to x-radiation which was similar to that of dividing cells, whereas the action of HN2 was different.

5. The ratios between b

solution of DNC instead of water, seedlings exposed to this solution behaved the same as seedlings treated with the same concentration of HN₂ made up in water.

Table 3 summarizes the data presented in the other tables. The mortality figures in line 2 show that, with the use of either method, corn root tissue with the greater amount of cell division present at the time of radiation was much more sensitive to x-rays than the same tissue with less cell division. In line 3 the figures from both methods

of Henshaw and Francis (4), who reported similar results with wheat seedlings. The fact that the roots whose rate of cell division was reduced by DNC were less sensitive to x-rays than normal roots of the same age is also in agreement with the general conception that sensitivity to radiation is usually directly proportional to the mitotic index of the tissue.

The experimental data presented here confirm the statement by Strong (7) that nitrogen mustard seemed to affect cells even before there was any

with similar tissue containing a higher percentage of dividing cells:

a) Embryos were germinated 12 hours for root tips containing no cell division and 16 hours for tips with about 15 per cent of the meristematic cells dividing.

b) Older seedlings were treated with 3,5-dinitro-*o*-cresol, which reduced the number of dividing cells in the root tip from the normal 38 per cent to 22 per cent.

2. When either method was used, the group of seedlings with the higher mitotic index was only slightly more sensitive to the effects of methylbis(β -chloroethyl)amine hydrochloride (HN₂) than the group with the lower index.

3. When either method was used, the group of seedlings which had the greater number of dividing cells at the time of exposure to x-rays was damaged significantly more than the group where there were fewer dividing cells.

4. Under the same experimental conditions, corn root tissue showed a significant increase in sensitivity to x-radiation with increase in the number of dividing cells, whereas no such relationship existed with the action of HN₂.

5. The ratios between biologically equivalent

doses of x-rays and a mustard compound may vary widely for the same tissue if its mitotic index is raised or lowered.

ACKNOWLEDGMENTS

We wish to acknowledge the courtesy of the Delaware Hospital which furnished the radiation.

REFERENCES

1. CLOWES, G. H. A., and KRAHL, M. E. Action of Dinitro Compounds on Sea Urchin Eggs. *Science*, 80:384-85, 1934.
2. FRIEDENWALD, J. S., and BUSCHKE, W. Studies on the Physiology, Biochemistry, and Cytopathology of the Cornea in Relation to Injury by Mustard Gas and Allied Toxic Agents. V. Nuclear Fragmentation Produced by Mustard and Nitrogen Mustards in the Corneal Epithelium. *Bull. Johns Hopkins Hospital*, 82:161-78, 1948.
3. GILMAN, A., and PHILLIPS, F. S. The Biological Actions and Therapeutic Applications of the β -Chloroethyl Amines and Sulfides. *Science*, 103:400-15, 1946.
4. HENSHAW, P. S., and FRANCIS, D. S. A Consideration of the Biological Factors Influencing the Radiosensitivity of Cells. *J. Cell. & Comp. Physiol.*, 7:173-95, 1935.
5. RUSSELL, M. A. Effects of X-Rays on Zea Mays. *Plant Physiol.*, 12:117-33, 1937.
6. ———. Nitrogen Mustard and X-Ray Research, pp. 1-7. Research Reviews (Office of Naval Research), May, 1950.
7. STRONG, L. C. Induction of Mutations by a Carcinogen. pp. 486-99. *Proc. 8th Internat. Cong. of Gen.*, 1948.

TABLE 4

COMPARISON OF THE EFFECT OF HN₂ ON NORMAL CORN SEEDLINGS AND ON SEEDLINGS TREATED WITH DNC TO REDUCE CELL DIVISION

EXP. NO.	HN ₂ dose 2.0 X 10 ⁻⁶ M (min.)	NORMAL CELL DIVISION (38 PER CENT)		CELL DIVISION REDUCED TO 22 PER CENT BY DNC	
		Controls	Treated	Controls	Treated
		No. of roots	Per cent roots stopped growing	No. of roots	Per cent roots stopped growing
1	16	17	0	40	17
2	18	18	0	30	63
3*	20	19	0	69	97
4	20	33	0	84	32
Total		88	2†	229	53†

* pH of water higher, HN₂ solution pH 6.6.

† Weighted averages.

TABLE 5

COMPARISON OF THE EFFECTS OF X-RAYS OR HN₂ WHEN APPLIED TO TISSUE WITH VARYING AMOUNTS OF CELL DIVISION OBTAINED BY TWO DIFFERENT METHODS

	METHOD A (TABLES 1 AND 2)		METHOD B (TABLES 3 AND 4)	
	16 15- or 16-hr. germinated embryos	0 11- or 12-hr. germinated embryos	38 Normal 48-hr. seedlings	22 DNC-treated 48-hr. seedlings
	No. of plants	Per cent mortality	No. of plants	Per cent mortality
Controls for radiation	142	3.5	140	0.7
Treated with X-rays	142	73.0	141	72
Treated with HN ₂	253	71.0	229	53
Controls for HN ₂	182	0	88	2

show that, when seedlings were exposed to HN₂, the variation in the amount of cell division at the time of exposure made no significant difference in the sensitivity of the roots. These results show that under the conditions of these experiments differences in mitotic activity during exposure affected sensitivity to HN₂ to a negligible degree, while the radiosensitivity of the same tissue increased with the amount of cell division present at the time of exposure to x-rays.

The data indicating a marked rise in sensitivity of embryo roots to radiation, at about the time that mitosis commences, agree with the findings

evidence of cell division. It may be that, although the seedlings showed the effects of x-rays and HN₂ in many similar ways, the action of the two agents was different when the reaction of resting cells was compared to that of dividing cells. Further experimentation may show whether this difference is mainly in the initial damage or whether the dividing cells are better able to recover after exposure to HN₂ than after x-ray treatment.

SUMMARY

1. Two methods were used to obtain corn seedlings with little or no cell division for comparison

combining them with the
cancer treatment—an area in
which a lot of research. They
are just after surgery, for in-
crease any stray cells that the
surgery has missed. They may
also with the targeted therapies
to stop tumour growth at the
source, often develop resistance
to therapies; hitting them
with a double-whammy might
be common.

The new treatments look promising.
But they do not look cheap. Yervoy costs
\$330,000 per patient per year, Opdivo
about \$150,000. As more drug companies
pile in, patients can hope that competing
drugs will hit the market soon. But as the
rich world grows older, fatter and therefore
more cancer-prone, one salient question is
how lucrative immuno-oncology might
prove for drug firms—and how affordable
for the insurers and governments that
would have to buy them. ■



ogy
op

blade arrangements make for faster helicopters

have clear advantages
over aircraft. But they also
have drawbacks: they are slow. The
fastest, the AH-64 Apache, can
exceed about 320kph
the cruising speed of
a propeller plane.
The AH-64 is in Florida,
making helicopter has
that may help to close
the gap. Two striking things
about the AH-64 Raider. The first is
that it is mounted one on top of
the other in opposite direc-
tions—much like the
coaxial rotors sold in toy
helicopters in the absence of a tail
rotor—a backwards-facing

complexity and the risk of the blades hitting
one another has made such machines un-
popular. Modern engineering methods, to-
gether with the use of stiffer carbon-com-
posite blades that are less likely to flex and
collide, promise to reduce such problems.

And twin rotors provide several advan-
tages. They offer inherent stability—which
is why children can fly the model choppers
equipped with them. The single set of ro-
tors on a standard helicopter produces a ro-
tational force, or torque. Without a tail ro-
tor to counter it, the fuselage would spin in
the opposite direction to the rotors. The
counter-rotating blades of the Sikorsky
machine cancel each other's torque.

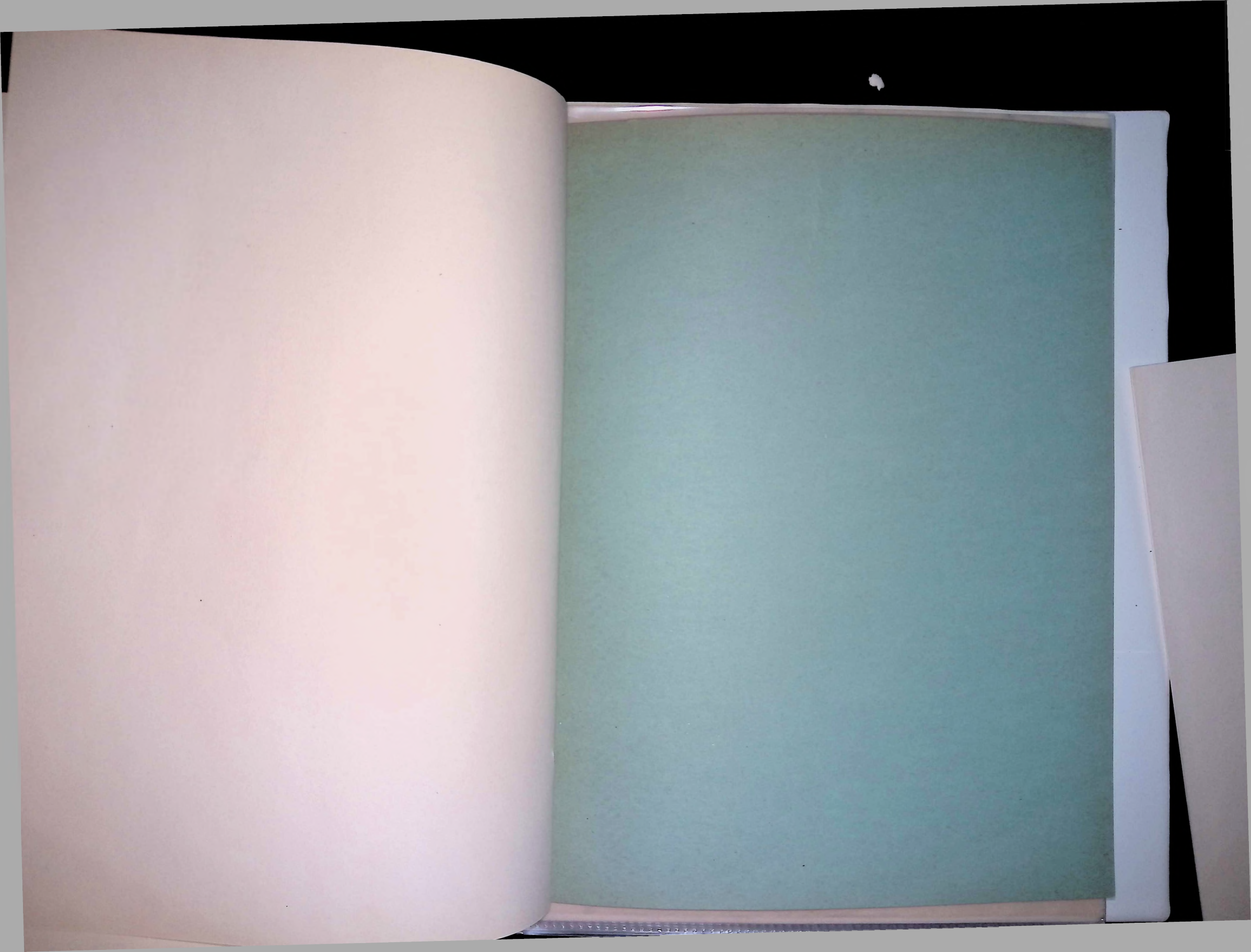
They also allow more speed. Like a
fixed wing, a rotor blade provides lift pro-
portional to the amount of air flowing over
it. In forward flight an advancing blade
moves faster, relative to the surrounding
air, than a retreating one. That creates un-
even lift that tries to roll the chopper over. ▶▶

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Combining them with the cancer treatment—an area in which a lot of research is being done—just after surgery, for instance, to kill any stray cells that the surgeon has missed. They may also combine the targeted therapies with the off-tumour growth at the site, often develop resistance to the therapies; hitting them with a double-whammy might be more effective.

The new treatments look promising. But they do not look cheap. Yervoy costs \$30,000 per patient per year. Opdivo about \$150,000. As more drug companies pile in, patients can hope that competing drugs will hit the market soon. But as the rich world grows older, fatter and therefore more cancer-prone, one salient question is how lucrative immuno-oncology might prove for drug firms—and how affordable for the insurers and governments that would have to buy them. ■



ogy
op

blade arrangements make for faster helicopters

have clear advantages over fixed-wing aircraft. But they also have drawbacks: they are slow. The fastest modern helicopter, the AH-64 Apache, exceeds about 320kph, the cruising speed of a modern propeller plane. The AH-64 is in service at a facility in Florida, making it the fastest helicopter that may help to close the gap between the two striking things about the AH-64: the first is mounted one on top of the other in opposite directions—much like the helicopters sold in toy stores in the absence of a tail rotor—a backwards-facing

complexity and the risk of the blades hitting one another has made such machines unpopular. Modern engineering methods, together with the use of stiffer carbon-composite blades that are less likely to flex and collide, promise to reduce such problems.

And twin rotors provide several advantages. They offer inherent stability—which is why children can fly the model choppers equipped with them. The single set of rotors on a standard helicopter produces a rotational force, or torque. Without a tail rotor to counter it, the fuselage would spin in the opposite direction to the rotors. The counter-rotating blades of the Sikorsky machine cancel each other's torque.

They also allow more speed. Like a fixed wing, a rotor blade provides lift proportional to the amount of air flowing over it. In forward flight an advancing blade moves faster, relative to the surrounding air, than a retreating one. That creates uneven lift that tries to roll the chopper over. ▶▶

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DEPARTMENT OF BOTANY

December 30, 1953

Examining Committee for

Mr. Francis J. Michellini

Gentlemen:

The Committee appointed to conduct the Ph. D. qualifying examination for Mr. Michellini is as follows: Professors Ralph O. Erickson, Chairman, John M. Fogg, Jr., D. R. Goddard (as an alternate, Dr. William Stepka), Olin E. Nelson, William Seifriz, and Conway Zirkle.

The exact date for the examination has not been set, but probably will be in the period between January 29 and February 5. The Chairman of the Committee will inform us regarding the time and place.

I hope each of you will be willing to serve on this committee.

Sincerely yours,



David R. Goddard

DRG/w

UNIVERSITY of PENNSYLVANIA

Department of Botany

May 12, 1955

TO: Drs. Edgar T. Wherry
Conway Zirkle
Edward C. Cantino
Herbert Stern
O. E. Nelson

This is to inform you of the final Ph.D. examination
of Mr. Francis J. Michelini on Tuesday, May 24, 1955 at 11:00 A.M.
at the Department of Botany, Macfarlane Hall.

For your convenience, a copy of the thesis will be
on file in the Department of Botany office after May 20.

David R. Coddard

dl

cc: F. Michelini

COPY

UNIVERS

The College

BOTANICAL LABORATORY
38th and Woodland Ave.

Dear Mr. Michelini:

A committee of the
making a careful study of
Pennsylvania. In their p
been selected for careful
of Botany, including the L
itself is cooperating in t
to aid the University.

The enclosed quest
out with complete franknes
Trustees Committee, but no
faculty will ever examine
any comment that you make
with your name.

The results from a
Executive Secretary's offi
so that we will have the c
up in any part of the stud
graduate students. This
dents in Botany and Microb
department in answering th
the University and by your
greatest value to us if yo
praise that part of the de
and refrain from criticism

May I take this op
participation in this eval

Mr. Francis J. Michelini
5612 Chew Street
Philadelphia 38, Pa.

UNIVERSITY of PENNSYLVANIA

PHILADELPHIA 4

The College

BOTANICAL LABORATORY
38th and Woodland Ave.

February 18, 1954

Dear Mr. Michellini:

A committee of the Board of Trustees of the University is making a careful study of the educational policy of the University of Pennsylvania. In their preliminary studies three departments have been selected for careful evaluation, one of these being the Department of Botany, including the Laboratory of Microbiology. The department itself is cooperating in this study, and each of you has an opportunity to aid the University.

The enclosed questionnaire, which we would like to have you fill out with complete frankness, will go to the Executive Secretary of the Trustees Committee, but no present or former member of the department faculty will ever examine your answers to the questionnaire, nor will any comment that you make concerning the department ever be identified with your name.

The results from all of the questionnaires, after study at the Executive Secretary's office, will be made available to the department, so that we will have the opportunity to correct weaknesses which are shown up in any part of the study, including that of the questionnaire of our graduate students. This letter is going to all current graduate students in Botany and Microbiology. Your aid to the University and the department in answering the questionnaire will be greatly appreciated by the University and by your department. In answering it you will be of greatest value to us if you will be completely candid and not merely praise that part of the department that you might have found satisfactory and refrain from criticism of our obvious shortcomings.

May I take this opportunity of thanking you in advance for your participation in this evaluation of the Department of Botany.

Sincerely yours,

David R. Goddard

David R. Goddard
Professor of Botany and
Chairman of Department

Mr. Francis J. Michellini
5612 Chew Street
Philadelphia 38, Pa.

UNIVERSITY INTRAMURAL CORRESPONDENCE

April 3, 1954

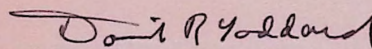
Francis J. Michelini

Dear Mr. Michelini:

I am happy to reappoint you as Assistant Instructor in Botany for the academic year 1954-55. I have every hope that you will complete your work for the doctor's degree during the course of the year.

This is the last appointment as Assistant Instructor that you can hold in the Department of Botany at the University of Pennsylvania. This is not because your services as Assistant Instructor have not been excellent, but because of the Department's belief that it is to the advantage of graduate students as well as to the Department that certain time limits be agreed to in the prosecution of graduate work.

Sincerely yours,



David R. Goddard
Professor and Chairman

DRG:r

cc - Dr. Erickson



Photo sent to me Nov. 8, 1970 (in picture).

1953-54 era photograph of Students and Department members during a field trip.

Dr. Goddard was my doctor and a speaker at my Inauguration in 1970.

Dr. Goddard - center with I. to r. back row - Joe Devlin, Dave Brown; Francis Michelini front row: Laboratory staff.

AL CORRESPONDENCE

1954

Assistant Instructor in
5. I have every hope that
doctor's degree during the

Assistant Instructor that
stay at the University of
your services as Assistant
but because of the Depart-
mentage of graduate students
ertain time limits be agreed
rk.

Sincerely yours,

David R. Goddard

David R. Goddard
Professor and Chairman



Photo sent to me Nov. 8, 2011 by fellow student Mel Belsky (not in picture).

1953-54 era photograph of University of Pennsylvania Graduate Students and Department Chairman Dr. David R. Goddard during a field trip.

Dr. Goddard was my doctoral thesis advisor in 1954 – 1955 and a speaker at my Inauguration as Wilkes President on Nov. 21, 1970.

Dr. Goddard – center with glasses
l. to r. back row – Joe Devlin behind the tree; Tom Humphreys;
Dave Brown; Francis Michelini; Milo Hyatt; Dave Hammond
front row: Laboratory staff - Connie Humphreys; Gloria Rosen

November 10, 2011

Dr. Melvin Belsky
[REDACTED]

Dear Mel:

Thank you so much for that photograph of the graduate students and Dr. Goddard. I think that was about 1953 or '54 on some field trip. The greenhouses do not look familiar. I grew my plants in the greenhouse behind the botany building but there was only ONE building, not the complex shown here.

I think the person hardly visible behind the tree on the left is Joe Devlin. The only one I can think of from that era standing behind Goddard would be Dave Brown. Remember him? His mother owned Nan Duskin women's hair styling and they owned a place on Long Beach Island at the shore where we went one weekend.

The lady you identified as Milo Hyat totally escapes my memory, unless she was our host?

I could not come up with the maiden name of Connie Humphreys. I know she and Gloria were the lab people for Goddard and she married Tom toward the end of his degree studies.

Lots of great memories from those years. I have been assembling memorabilia from my life in plastic envelopes in three ring binders. Makes it easy to organize stuff when I find more stuff. I think of Jerry Schiff often. He helped me so much in the lab when I was struggling with the gas exchange techniques. I still think of the basement kitchen cooking club from '50 - '51 before I married Anne Marie and had home cooking!

It is always great to hear from you. Will try to get to NY in the spring, meanwhile keep in touch.

Mike

The photos on the foll
at the University of Pe
taken during a field tri
renowned plant physic
but David Rosen appe



Dave Hammond, Joe Dev

November 10, 2011

photograph of the graduate
that was about 1953 or '54 on
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n.

The photos on the following pages were taken during the 1951/1952 academic year at the University of Pennsylvania. It appears to me 64 years later, (2016), they were taken during a field trip for a course being taught by Professor William Seifriz, a renowned plant physiologist of the day. Participants were primarily graduate students but David Rosen appears to be leading the field trip.



Joe Devlin, Hosoi, Mike Michelini, Tom Humphrey



Dave Hammond, Joe Devlin, Hosoi, Tom Humphrey, Mike Michelini, Helen Stafford



Gloria & Dave Rosen, Helen Stafford, John Wallace



Dave Hammond, Joe Devlin, Connie Holden, Hosoi, Tom Humphrey, Mike Michelini, Helen Stafford



Peter Sax, Hosoi, Professor Seifriz, Louie Lukens, John Wallace

The photos on the following
at the University of Pennsylv
taken during a field trip for a
renowned plant physiologis
but David Rosen appears to



Lukens, Professor Seifriz, Connie



L to r: Connie Holden, Rosemarie
Stafford, Dave Hammond (in rear
head)



Stafford, John Wallace



oi, Tom Humphrey, Mike Michelini, Helen Stafford



Louie Lukens, John Wallace

The photos on the following pages were taken during the 1951/1952 academic year at the University of Pennsylvania. It appears to me 64 years later, (2016), they were taken during a field trip for a course being taught by Professor William Seifriz, a renowned plant physiologist of the day. Participants were primarily graduate students but David Rosen appears to be leading the field trip.



Lukens, Professor Seifriz, Connie Holden



David Sax & Louie Lukens



L to r: Connie Holden, Rosemarie Wilford, Gloria Rosen & baby, Dave Rosen, Helen Stafford, Dave Hammond (in rear), John Wallace (on ground), F. J. Michelini (back of head)



Facing camera: Tom Humphrey, Joe Devlin, John Wallace, Louie Lukens (bending over), Mike Michelini (leaning over fire)



Helen Stafford, Rosemary Wilford, Gloria Rosen, Connie Holden, Louie Lukens, Peter Sax & Dave Hammond (foreground)



Mike Michelini, (?)



Peter Sax, Lecturer,
Joe Devlin (back), Da



L to r: Rosemary Wilford, Dave Ha
Joe Devlin, Kathy Sax (in front of
Peter Sax



hrey, Joe Devlin, John Wallace, Louie Lukens (bending
ng over fire)



Wilford, Gloria Rosen, Connie Holden, Louie Lukens,
d (foreground)



Mike Michelini, (?)



Peter Sax, Lecturer, Rosemary Wilford, Gloria Rosen, Connie Holden, Louie Lukens,
Joe Devlin (back), Dave Hammond & John Wallace (kneeling)



L to r: Rosemary Wilford, Dave Hammond, Professor Seifriz, Gloria Rosen in front of
Joe Devlin, Kathy Sax (in front of Hosoi), Dave Rosen (in front of Louie Lukens),
Peter Sax

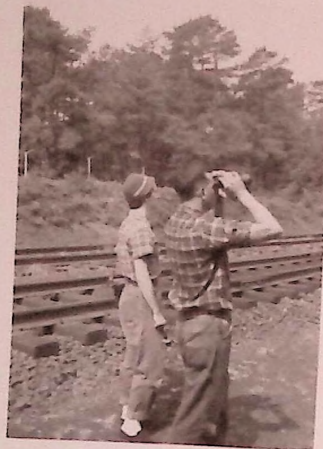


Top right: Mel & Charlotte Belsky; Anne Marie Sterner

Sitting on track: Charlotte, Anne Marie, Mike Michelini

Lower right: Polly Mons, Anne Marie & Mike Michelini

Below: Polly Mons & Mike (Birding)
Mike & Anne Marie



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Photos of
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Photos of Mike and Anne Marie with Mama and Papa Sterner after Ph. D. commencement from U. of Pennsylvania in May of 1955

Photo at June 1955 Commencement from University of Pennsylvania
 Left photo: Mama & Papa Michelini with sister Lucia and brother
 August

Right photo: Anne Marie & Francis Michelini with Francis & Anna
 Sterner, her parents



Professor H.
 Department
 California I
 Pasadena, Ca

Dear Dr. H.

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University of Pennsylvania
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ni with Francis & Anna



Professor Ralph O. Erickson
Department of Biology
California Institute of Technology
Pasadena, California

January 17, 1955

Dear Dr. Erickson:

It may come as a shock to you but I am still alive and in attendance at the University of Pennsylvania. I hope you will forgive my lack of correspondence as due to a very full program and not just plain laziness. In part it is due to the complete unselfishness on Dr. Goddard's part for he has been a tremendous help whenever the inevitable problems have arisen. Even now I am writing more in terms of a progress report and the estimated time schedule which both he and I hope to follow, rather than with problems.

The chlorophyll data stands much as you saw it with the suggested additional treatments you had suggested. This semester has seemed to just fly by in retrospect, especially when I think of what is still left to do. I do have the slides of the apices of each of the three leaves, 5, 9, 13, from a plastochron age of minus 3 to plus 1. I hate to say this but so far they are very disappointing in terms of any critical chloroplast studies. I have the material in three different fixatives so I doubt whether it is due to cytological technique. The chloroplasts themselves just seem very small and difficult to identify with any degree of certainty. At the moment, Dr. Goddard has suggested I keep them in abeyance and concentrate on the respiration and photosynthesis studies. After a considerable amount of time, during which I followed Dr. Einstein's advice and learned some plumbing, I got the Penn rewired, heated, lit, and refrigerated, as well as cast into darkness. The latter became necessary after it became apparent that it was technically impossible to measure respiration and photosynthesis in the same material by changing the conditions in the vessel. Respiration required KOH in the sidearm to absorb CO₂ while the photosynthesis required an O₂ absorber. The vessels have to be thoroughly cleaned between each, so it was decided to measure them separately and plot each against plastochron. The respiration has worked out fairly well so far but in measuring photosynthesis I've been measuring some reaction of the phosphorous we are using as the reductant in the system. We've tried everything we can think of to clear it up but so far, nothing. Dr. Goddard is going to spend tomorrow morning in the lab with me and see if we can pin it down, if not, we'll have to make some changes in the system.

That, in a nutshell, is the present state of affairs. Dr. Goddard would like to see me graduate in June, and, needless to say, so would I. I've been proceeding with some of the writing of Materials and Methods section in hopes that we will lick the photosynthesis and I can get some decent measurements in a few days.

I hope I haven't sounded too pessimistic but it sure can get you down at times. Dr. Goddard says that for a morphologist I'm turning into not a half-bad physiologist but I feel certain I could give him a pretty good argument. I do feel as though I'm learning a tremendous amount both technically and theoretically with respect to physiology and as a background for teaching I can't begin to place a value on the experience.

Enough of me now. I hope your stay has been all you wished and that your family is enjoying the California environs. We have a post-doctoral fellow here from UCLA in Berkeley and as with most Californians he is constantly singing its praises.

Please give my regards to your wife and girls. I can promise I won't let much time elapse before you hear from me again. I just hope it will be good news on progress then.

Sincerely,

Francis J. Micholini

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Dr. P.
Hosoc
May 11

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has been all you wished
the California environs.
from UCLA in Berkeley
constantly singing its

and girls. I can promise
you hear from me again.
progress then.

Sincerely,

Francis J. Michelini

March 11, 1955

Dr. Philip R. White
Boncoe B. Jackson Memorial Laboratory
Bar Harbor, Maine

Dear Dr. White:

Dr. Goddard has informed me of your suggestion
that I write and acquaint you with my background and
interests. I have enclosed a vita which I think will
cover my background in science.

As Dr. Goddard has informed you my training
has not been primarily in physiology but rather in
cytology, morphology and developmental studies. My
early graduate research at the University of Delaware
involved considerable cytology while my graduate
work at the University of Pennsylvania has involved
a certain amount of physiology. My thesis work has
been done under the direction of Dr. Erickson and is
primarily a developmental study using a morphological
index. This index was developed by Dr. Erickson and
I and reported at the A.I.B.S. meetings at Cornell in
1952. I have studied increases in fresh weight, dry
weight, chlorophyll content, and respiration in leaves
at all stages from primordia to maturity. This latter
work has been done under the direction of Dr. Goddard.

I do think that the program you outlined in
your original letter sounds very interesting and I
would like to be considered by you for this position.
I can assure you that I would make every effort to
acquire any additional knowledge that you might consider
essential in the position.

I hope I may be able to talk to you when you
visit Philadelphia in April. Thank you very much for
your interest.

Sincerely yours,

Francis J. Michelini
Assistant Instructor

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UNIVERSITY OF ARKANSAS
COLLEGE OF ARTS AND SCIENCES
FAYETTEVILLE

DEPARTMENT OF BOTANY
AND BACTERIOLOGY

September 15, 1955

Dr. Francis J. Michellini
Wilkes College
151 Alexander Street
Wilkes Barre, Pennsylvania

Dear Dr. Michellini:

Your note suggesting a meeting at the Smoker was handed to me just as Mrs. Swartz and I were checking out of the Kellogg Center about 1:30 P.M. Due to the opening of school and other duties, it was necessary for us to start back to Arkansas a little early. Being a relatively slow driver, it takes me more time to get home than it would members of the younger generation.

Seriously, I am sorry to have missed you, but couldn't figure out how to contact you on short notice. Before I left here, I checked your paper, and planned to hear it, but couldn't get to the meetings at that time. I had received very fine letters concerning you and wanted to meet you. Of course, Dr. Goddard spoke very highly of you. Perhaps we can meet some time during the year.

Our registration has just been completed and our classes are overflowing. It looks like we may have to add additional men next year. If you are still interested in coming here, I would suggest that you write to me about it during early January. By that time plans will be under study. I have placed your papers in our possible personnel file. Don't hesitate to write me at any time.

My visit at Michigan State was quite revealing. It certainly has changed since I was a student there in 1924. I had a difficult time finding my way around.

I am sorry to have missed you. Thanks for your note.

With all good wishes, I am

Very truly yours

Delbert Swartz
Delbert Swartz
Chairman

DS:all

Dr. H.A. Russ
Dept. of Biol
University of
Newark, Dela

Dear Dr. Russ

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Michellini! I
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OF ARKANSAS
RTS AND SCIENCES
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15, 1955

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duties, it was necessary for us to start
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I you, but couldn't figure out how to
I left here, I checked your paper, and
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ns will be under study. I have placed
l file. Don't hesitate to write me at

revealing. It certainly has changed
I had a difficult time finding my way

ks for your note.

Very truly yours

Delbert Swartz
Delbert Swartz
Chairman

May 26, 1955

Dr. M.A. Russell
Dept. of Biological Sciences
University of Delaware
Newark, Delaware

Dear Dr. Russell:

I have been waiting to do this for some time now but it
always seemed as if there were one more hurdle to get by before
I could take time out to write you. It seems that after five
years I'm by then all for Dr. Goddard now addresses me as Dr.
Michelin! I took my final Tuesday morning and in all honesty
I can say that it went very well. Dr. Goddard was especially
pleased and has really been wonderful to me. Tell Arnold Clark
that everything has worked out fine and I still appreciate
tremendously his pre-prelim visit. Delaware people will always
be the roots of the tree of my training.

Now some news. I was offered a job by Philip White in
his laboratory at Bar Harbor but turned it down. It was against
Dr. Goddard's advice and I had qualms at the time too but I
had to make the decision as I saw best and that was it. I had
decided before that that I wanted to make my niche in teaching
and I feel the sooner I get the experience in that line the
better prepared I'll be for the kind of job I'm looking for in
3 or 4 years when teachers will really be in demand. After I
turned down the White job an offer came from a small college
in Wilkes-Barre, Pa. to teach biology and histology as an
assistant professor at \$4500 for ten months. It isn't what I
want as a permanent position for there won't be much opportunity
to do research but it will be the kind of training I want and
the experience in biology will be broadening after so much botany.
There were lots of pros and cons but I think it will work out.
I go up for a visit next Tuesday and I'll know more of the details
then. I hope to get to Delaware possibly the following week if
you plan to still be around. Could you drop me a line and let me
know what a good time the week of the 6th would be?

The thesis ~~work~~ worked out wonderfully. Goddard was very
impressed and said I should give a paper at the A.I.B.S. meetings
at East Lansing in September so I'm planning to attend. Will you
be going? and if you are would you be interested in a ride? I'm
also going to be looking for a job for the following year by
talking to people and letting them know I expect to be available
for 1956. I would like a state university with enrollment of
about 3000 to 4000 eventually. If you hear of anything.....
I didn't forget your very kind offer of the use of your gown for
commencement. I received a very nice gift from my mother and father
for the purchase of a cap and gown for a graduation present so
Thank You very much for your thoughtfulness. See you soon, Love, Mike



is long enough, I'd feel
honored for you to wear it
when your turn comes.

I hope Ann Marie feels
proud of her part in this too -
for I'm sure the "bride" had
to entertain herself very often
when you had to study.

Come down soon -

And my best,

Mary Russell.

Feb. 1 -

Dear Mike -

I'm proud of you! For
me the prelims were the
biggest job of all, because
in the finals your field's
narrowed to things related
to your thesis (at least
theoretically). Mary Medill
gave us an interesting
seminar the other day &
took my cap - gown & hood
back with her. I'd the gown

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STAPLES

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STAPLES

STAPLES

WILKES COLLEGE
WILKES-BARRE
PENNSYLVANIA

May 23, 1955

Mr. Francis J. Michelini
Botanical Laboratory
38th and Woodland Avenue
Philadelphia 4, Pennsylvania

Dear Mr. Michelini:

Dr. Reif and I were delighted to hear that you plan to be with us next year and we are looking forward to an association which we hope will be happy on both sides.

A Memorandum of Agreement is enclosed which I hope you will sign and return or bring with you to Wilkes-Barre on Tuesday, May 31, or Thursday, June 2. At that time I will sign both copies so that our agreement may be complete.

Dr. Reif indicates that he will hold both of the above dates open for you and will plan to see you in the afternoon of whichever day you plan to visit us.

Best wishes to you.

Very sincerely yours,

Eugene L. Esf
President

ESF/js
Enclosure

WILKES COLLEGE
WILKES-BARRE
PENNSYLVANIA

May 23, 1955

MEMORANDUM OF AGREEMENT

It is mutually agreed between Wilkes College and Francis J. Michelini that he shall serve as Assistant Professor of Biology from September 1, 1955, through June 30, 1956.

For his services Mr. Michelini shall receive \$450 per month.

Francis J. Michelini
Francis J. Michelini

Eugene S. Farley
Eugene S. Farley

SIGMA XI, THE SCIENTIFIC RESEARCH SOCIETY

Francis J. Michelini

Member ID: 550090132
Full Emeritus Member
Wilkes University
Active Member 7/1/2010 through 6/30/2011
Elected to Membership in 1955

COPY

Miss Ware
School and
University
Philadelphia

Dear Miss

I
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I
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COLLEGE
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1955

OF AGREEMENT

Wilkes College and Francis J.

Assistant Professor of Biology

June 30, 1956.

shall receive \$450 per month.

Francis J. Micheline
Francis J. Micheline

Eugene S. Farley
Eugene S. Farley

COPY

May 26, 1955

Miss Marjette Quick
School and College Placement Service
University of Pennsylvania
Philadelphia, Pennsylvania

Dear Miss Quick:

I have enclosed a check for \$1.00 in payment for transmitting one set of credentials to Dr. Delbert Swartz, University of Arkansas. Thank you very much for your service.

I would also like to inform you that I have accepted a job that was brought to my attention by the placement service. It is as an assistant professor in the biology department at Wilkes College, Wilkes-Barre, Pa. I wish to thank you very much for your part in obtaining this position. The contract for the position is for 10 months and I do expect to be looking for another position at this time next year. I would appreciate it if you would keep my file open with respect to jobs for September 1956. I am hoping to ultimately find my niche in a small state university of 3000 to 5000 enrollment in the Eastern United States. I know this sounds optimistic at this time but I feel with my botanical training and the experience I will have gained in teaching biology for a year or two I might find such a position. I would appreciate it very much if you would keep me in mind in the event such an opening might come to your attention. This request is a long range one, 3 or 4 years probably, but I will try to keep my file as up to date as possible.

Thank you very much for your help in the past and your interest in the future.

Sincerely yours,

Francis J. Micheline
Assistant Instructor

*The Classes of
Nineteen Hundred and Fifty-five
of the
University of Pennsylvania
announce their
Graduation
on Wednesday June fifteenth
Philadelphia*

UNIVERSITY of PENNSYLVANIA

ONE HUNDRED AND NINETY NINTH

Commencement

Wednesday Morning, June 15, 1955
Ten Fifteen O'clock

Municipal Auditorium
Thirty Fourth Street below Spruce Street

ADMIT BEARER

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ITEM#81018
BETTER office products CANOGA PARK, CA 91304

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PENNSYLVANIA

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June 15, 1955

O'clock

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low Spruce Street

Reprinted from the AMERICAN JOURNAL OF BOTANY, Vol. 44, No. 4, 297-305, April, 1957
Printed in U. S. A.

from the files
of F. J. Michelini
"Dr. Mike"

THE PLASTOCHRON INDEX

Ralph O. Erickson and Francis J. Michelini

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BETTER ITEM#81018
office products CANOGA PARK CA 91304

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THE PLASTOCHRON INDEX

Ralph O. Erickson and Francis J. Michelini

THE PLASTOCHRON INDEX

Ralph O. Erickson¹ and Francis J. Michelini

IN DEVELOPMENTAL STUDIES one can usually relate only the simplest aspects of the developing organism or organ to time directly. For instance, if one were studying a developing leaf, one could measure its length or width on successive days without injuring it, but many kinds of study which one might wish to make are destructive. Even such a simple measurement as its dry weight cannot be obtained without killing it, and one obviously cannot cut microtome sections of the same leaf more than once. This circumstance applies to almost any study of growth and development, and one must deal with parallel specimens or samples, each of which is sacrificed at a given time to provide the required data. Whether the data are obtained by histological examination, chemical analysis, metabolic study, or other means, they must be related to time if they are to have meaning in developmental terms. In many cases, plotting the observations directly against the chronological age of the plants is of limited value. Unless one is working with genetically uniform plants under exactly controlled conditions, variability may be so great that plants of the same chronological age may have reached quite different stages of development, while plants which are morphologically similar may be of quite different chronological ages. Under these conditions, significant relationships may be obscured, even when the statistical precautions of taking large samples and randomizing the error are observed.

These difficulties can often be minimized by using a numerical *index* of the developmental status of each specimen and relating the observations to time indirectly by way of the index. This developmental index should be based upon measurements of the plant which can be made easily and without injury, and its relationship to time should be worked out by appropriate growth studies. Observational data can then be plotted against the developmental index of each plant used, and the resulting graphs interpreted, on the basis of the growth studies, as a plot of the data in question against time.

The advantages of this procedure are illustrated by studies of developing flower buds and anthers of *Lilium* (Erickson, 1947, 1948; Ogur et al., 1951). The logarithm of length of the flower bud was shown to be linearly related to time, and

served as a developmental index for organs and processes within the flower. By relating various measurements and observations to this index, growth curves were constructed and time rates of change were calculated for features of the flower bud and anther which could not have been followed through time by direct means. In addition, this technique served to circumvent the environmental variability of the material to a considerable extent. Although there were large fluctuations in the daily increment of log bud length, the relationships of anther dimensions, and of meiosis and microspore mitosis, for instance, to log bud length were very close indeed.

In the shoot of a higher plant, leaves appear periodically, and the period between initiation of successive leaves has been termed a plastochron. This term was proposed by Askenasy (1880) to designate the interval of time between formation of two successive internode cells in *Nitella flexilis*. He also proposed that the term might be used for all plants having a similar nodal organization, and the concept of the plastochron has gained fairly wide currency, particularly in shoot apex studies of the higher plants (e.g., Esau, 1953). When successive plastochrons are equal in duration, the plastochron may be made to serve as the unit of a developmental scale. A plastochron index for shoot development in *Xanthium* is described below. This index is linearly related to time and serves to specify the developmental status of a shoot with an error of only a few hours. The plastochron index has points in common with procedures which have been used by other authors in physiological and morphological studies of shoot development, as will be pointed out in the discussion.

GROWTH MEASUREMENTS.—The argument presented in this paper is based on growth observations made on *Xanthium* plants. Burs were collected in Delaware County, Pennsylvania, from plants growing as weeds, which should probably be assigned to *X. italicum* Moretti.² Seeds were removed from the burs, scarified to insure germination, and planted fortnightly in pots. The plants were kept in a lighted greenhouse and repotted at intervals. Solar illumination was supplemented before dawn and after dusk so as to give the plants 16 hr. of light per day. Since *Xanthium* is a short-day plant, it can be kept in strictly vegetative growth by this regime. The total length, including

¹ Received for publication August 14, 1956.

During a part of the time that this paper was under consideration, the senior author held a fellowship of the John Simon Guggenheim Memorial Foundation and enjoyed the facilities of the Earhart Plant Research Laboratory at the California Institute of Technology.

² We are indebted to Dr. Herbert Wahl for identifying our plants. They do not appear to differ in growth characteristics from the strain used by K. C. Hammer and other workers on photoperiodism, which is usually referred to as *X. pennsylvanicum*.

lamina and petiole, of each leaf on each of 20–30 plants was measured daily at approximately 10 A.M. The smaller leaves were measured to the nearest 0.1 mm. with a vernier calipers, and larger ones to the nearest 1 mm. with a rule.

DERIVATION OF THE PLASTOCHRON INDEX.—An inspection of the sigmoid growth curves of the leaves suggests that they may be regarded as exponential in their early portion, as Blackman (1919) and other authors have found for many plants and plant organs. This supposition is supported by plotting leaf length logarithmically against time, as in fig. 1, where the relationship between log leaf length and time for one plant appears to be linear up to about 70 mm. leaf length. Other plants grew in essentially the same manner. Note that we have numbered the leaves serially in order of their appearance in time.

Geometrically considered, the lower portion of fig. 1 may be regarded as a family of straight lines.³ It is further evident that the lines are approximately parallel, and, with the exception of those for the cotyledons and leaves 1 and 2, that they are approximately equally spaced along the abscissa.

A plastochron is conventionally defined as the time interval between initiation of two successive leaves. It might be more broadly defined as the interval between corresponding stages of development of successive leaves, and one might choose initiation, maturity, or any intermediate stage of development as the stage of reference. For the present purpose, we shall define the reference stage in terms of a measurement of leaf length. A length of 10 mm. is convenient for the *Xanthium* data, since leaves of this order of length are growing exponentially, and yet are large enough to be measured with some accuracy and without injury to the shoot.

In terms of this reference length, the logarithm of which is indicated as a horizontal line in fig. 1, a plastochron may be visualized as the distance between the intersections of the reference line with the growth curves of two successive leaves. The "developmental age" of a plant may be defined arbitrarily in terms of plastochrons conceived in this way. Specifically, we shall regard a plant as being exactly n plastochrons old when leaf n is 10 mm. long, $n + 1$ plastochrons old when leaf $n + 1$ is 10 mm. long, and so on. When, however, no leaf is exactly 10 mm. long, an exact specification of

³ Actually, the leaves exhibit a marked diurnal rhythm in growth rate. From a limited series of hourly photographs of leaves growing at a constant temperature of 23°C. with alternating periods of 16 hr. of illumination and 8 hr. of darkness, it is clear that the rate of elongation in the dark may be twice as great as during the light period. In one case, a leaf about 50 mm. long varied in its rate of elongation from about 0.4 mm. · hr.⁻¹ in the dark to 0.2 in the light. We have not considered in detail the implications of this periodicity in calculations of the plastochron index, but we feel that it can be ignored in most studies in which interest does not center on direct periodic effects.

the plastochron age of the shoot requires interpolation. If, for instance, leaf 5, n , were 18 mm. and leaf 6, $n + 1$, 5 mm. long at a given time, it is clear that the plant would be more than 5 but less than 6 plastochrons old. A method for interpolation can be based on the assumptions that the early portions of the logarithmic growth curves are (1) linear, (2) parallel, and (3) equally spaced, as stated above.

Figure 2 is a diagrammatic representation of a portion of fig. 1 in which the diagonal lines represent portions of the leaf growth curves, and the horizontal line is drawn to correspond to the reference length, 10 mm. A vertical line is drawn at a particular time, when the plant is more than n but less than $n + 1$ plastochrons old. Since the plastochrons are assumed to be equal in duration, the plastochron age at this time, by linear interpolation, is $n + (AB/AC)$. This interpolation, however, is dependent on knowing the leaf lengths before and after the time in question. If the plant were sacrificed for study at that time, no growth data would be obtainable thereafter, and, in any case, the collection of growth measurements for a large series of plants is laborious. Since triangles ABD and CBE are similar, it follows that $AB/AC = DB/DE$, and that the plastochron age can equally well be estimated as $n + (DB/DE)$. Since the points D , B , and E represent logarithms of leaf length, the plastochron age can be rewritten

$$P.I. = n + \frac{\log L_n - \log 10}{\log L_n - \log L_{n+1}} \quad (1)$$

where n is the serial number of that leaf which just exceeds 10 mm. in length, and $\log L_n$ is the logarithm of length in mm. of leaf n . We shall refer to this expression as the "plastochron index" (*P.I.*), or the plastochron age of a shoot. To calculate it, it is only necessary to count the leaves on the shoot which are longer than 10 mm. and to measure the lengths of the two successive leaves which are respectively just longer and just shorter than 10 mm. at a given time. Logarithms to any base may be used, but the use of natural logarithms leads to an easy method of slide-rule calculation, and has advantages in the growth rate considerations presented below.

A second method of derivation of the plastochron index may be illustrated by replotting the data of fig. 1 as in fig. 3. Here each curve is a graph of log leaf length against node number at a given time. Again, a family of curves appears, which may be regarded as linear at lower values, parallel and equally spaced. However, the lines have negative slopes. In a diagrammatic representation of part of one of these curves, fig. 4, the same relationships shown above may be verified geometrically

$$P.I. = n + \frac{AB}{AC} = n + \frac{DB}{DE} = n + \frac{\log L_n - \log 10}{\log L_n - \log L_{n+1}}$$

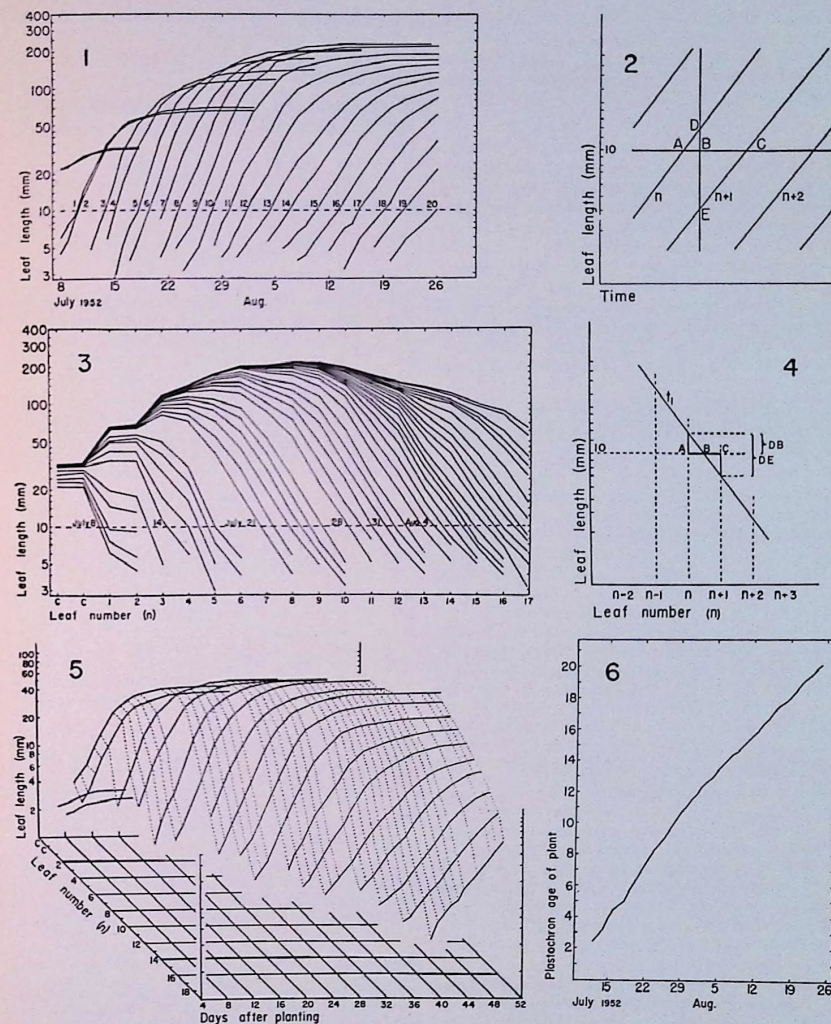


Fig. 1-6.—Fig. 1. Lengths of successive leaves of a *Xanthium* plant plotted logarithmically against time. Each growth curve applies to a single leaf. The unmarked curves at the left apply to the cotyledons.—Fig. 2. Idealized geometric representation of a portion of fig. 1.—Fig. 3. Leaf lengths of fig. 1 plotted logarithmically against node number. Each curve applies to a single day.—Fig. 4. Geometric representation of a portion of fig. 3.—Fig. 5. Leaf lengths of fig. 1 and 3 plotted logarithmically against time and node number.—Fig. 6. Plastochron index, calculated from the data of fig. 1, plotted against time.

These relationships may also be developed from a third point of view, based on growth considerations. If the early portion of the growth curve of a leaf is assumed to be exponential, its equation may be written

$$L = L_0 e^{rt} \text{ or } \ln L = \ln L_0 + rt \quad (2)$$

where t is time, L is length of a leaf, L_0 is length of that leaf at $t = 0$, e is the base of natural logarithms, $\ln$ is the natural logarithm, and r is the relative rate of elongation of a leaf, dL/Ldt . If it is assumed that r is constant for a number of successive leaves, equation (2) may be made to describe the early elongation of each of these leaves by changing only the value of L_0 for each. On the assumption made above that leaves appear at equal intervals of time, the changes in L_0 for successive leaves will be regular. Equation (2) may be written specifically for the two successive leaves n and $n+1$

$\ln L_n = \ln L_{n0} + rt$ and $\ln L_{n+1} = \ln L_{(n+1)0} + rt$ and rt may be eliminated between the two equations, giving

$$\ln L_n - \ln L_{(n+1)0} = \ln L_n - \ln L_{n+1} = p$$

This implies that in applying (2) to the second of two successive leaves, the value of $\ln L_0$ used for the first leaf should be decreased by an amount equal to the difference between the natural logarithms of lengths of two successive leaves. For leaf $n+2$, twice this difference should be subtracted, and so on for any number of successive leaves. On the three assumptions we have made (p. 5) this difference is a constant. It is the natural logarithm of the ratio of lengths of two successive leaves and will be symbolized p . A more general equation may now be written, expressing the length of any leaf during its early growth as a function of its serial number and time

$$\ln L = \ln L_0 + rt - pn \text{ or } L = L_0 e^{rt-pn} \quad (3)$$

where p is a constant equal to $(\ln L_n - \ln L_{n+1})$ or $\ln(L_n/L_{n+1})$, and n is the serial number of a given leaf, which can be only a positive whole number. Equation (3) suggests an isometric representation of the data of fig. 1. This is drawn in fig. 5, where log leaf length is plotted against time and node number. The solid lines in this figure correspond to the curves drawn in fig. 1, and the dotted lines through the same points to those of fig. 3.

The plastochron index may now be derived from (3). It should first be noted that the plastochron index ($P.I.$) scale corresponds to that of leaf number (n), with the difference that n is discontinuous and $P.I.$ is visualized as a continuous variable. $P.I.$ and n coincide at the integral values 1, 2, 3, . . . but $P.I.$ can take intermediate values as well. In this sense $P.I.$ may be substituted for n in (3). The plastochron index can then be formally defined as that value which satisfies (3) for the reference length at a given time. Substituting $L = 10$ into (3) and rearranging

$$P.I. = \frac{\ln L_0 + rt - \ln 10}{p} \quad (4)$$

One may add $(pn - pn)$ to the numerator on the right, substitute $\ln L_n$ from (3) and rearrange to obtain

$$P.I. = n + \frac{\ln L_n - \ln 10}{p}$$

Since $p = \ln L_n - \ln L_{n+1}$

$$P.I. = n + \frac{\ln L_n - \ln 10}{\ln L_n - \ln L_{n+1}}$$

which is identical with (1).

Calculation of the plastochron index may be illustrated with a portion of the data plotted in fig. 1 (table 1). This plant was started from seed on 1 July and the first leaf measurements were made on 8 July. The two leaves used as a basis of calculation each day are italicized. On 24 July, for instance, leaf 8 was longer and leaf 9 shorter than 10 mm. The integral part of the index is therefore 8. The fractional part is most easily evaluated with a log-log slide rule as follows

$$P.I. = 8 + \frac{\ln(12.7/10)}{\ln(12.7/6.1)} = 8 + \frac{\ln 1.27}{\ln 2.08} = 8 + \frac{0.239}{0.733} = 8.33$$

A consideration of the various equations above will suggest other ways of estimating $P.I.$ from leaf length measurements.

Rearranging (4) gives

$$P.I. = \frac{\ln L_n - \ln 10}{p} + \frac{r}{p} t \quad (5)$$

indicating that $P.I.$ is a linear function of time, with r/p as the slope and $(\ln L_n - \ln 10)/p$ as the ordinate intercept. In connection with (2) above, it was noted that r is the relative rate of elongation of a leaf, dL/Ldt , and it might also be written $d \ln L/dt$. The term p is analogous, and might be termed the relative plastochron rate of elongation.⁴ Since n is discontinuous, however, p cannot properly be represented as a derivative. It might be written $\Delta \ln L/\Delta n$, t being held constant. In exponential growth, r may similarly be written $\Delta \ln L/\Delta t$.

⁴It is of interest to note that Askenasy (1880) calculated values of "Wachstumsgeschwindigkeit" as the natural logarithm of the ratio of lengths of two successive internodes, and symbolized it as a . We feel that this expression, which is equivalent to our p , the relative plastochron rate, may have great morphological interest. In exploratory work with *Xanthium*, which the senior author has done at the Earlhart Plant Research Laboratory, it appears that p is relatively invariant with changes of temperature, whereas r , the relative time rate of elongation of the leaves, varies markedly with temperature. Furthermore, it is clear that the value of p for various dicotyledonous species varies widely, and we have preliminary data which suggest that p may have some relation to the phyllotactic divergence of the leaves.

TABLE 1. Leaf lengths of a *Xanthium* plant and values of the relative plastochron rate (p), the plastochron index ($P.I.$), and leaf plastochron index ($L.P.I.$) on successive days

Date	Leaf number (n)										p	$P.I.$
	3	4	5	6	7	8	9	10				
	Leaf length (mm.)											
July												
21	52	54	40.4	18.6	8.5	4.1					0.784	6.79
22	55	60	42	30	12.7	5.8					0.786	7.30
23	57	64	54	45.2	19.9	9.0	4.9				0.795	7.87
24	57	67	62	47	29.4	12.7	6.1	3.2			0.733	8.33
25	58	70	67	58	40.3	17.6	7.5	4.0			0.853	8.66
26	59	70	72	42	42	24.7	11.1	5.1			0.805	9.13
	Leaf plastochron index											
21	3.79	2.79	1.79	0.79	-0.21	-1.11						
22	4.30	3.30	2.30	1.30	0.30	-0.70						
23	4.87	3.87	2.87	1.87	0.87	-0.13	-1.13					
24	5.33	4.33	3.33	2.33	1.33	0.33	-0.67	-1.67				
25	5.66	4.66	3.66	2.66	1.66	0.66	-0.34	-1.34				
26	6.13	5.13	4.13	3.13	2.13	1.13	0.13	-0.87				

Δt . Taking equal values of the numerators of the two expressions

$$\frac{r}{p} = \frac{\ln L/\Delta t}{\ln L/\Delta n} = \frac{\Delta n}{\Delta t}$$

that is, the rate of change of node number with time, or the rate of appearance of leaves. If Δn is taken as unity, Δt will be the time required for two successive leaves to pass the reference length. That is to say, p/r corresponds to the average duration of a plastochron.

The linearity of the relationship between the plastochron index and time can be tested by plotting $P.I.$ as calculated above against days. As shown in fig. 6, a satisfactory straight line results for a considerable period. As in many dicotyledonous seedlings, leaves 1 and 2 are nearly opposite and of nearly equal lengths throughout their development. That is to say, plastochron 1, as we define it, is very short or zero. Hence it is useless to calculate a plastochron index until leaf 2 has exceeded 10 mm., i.e., until the plant has entered the second plastochron. It will also be noted from fig. 1 that plastochron 3 is shorter than either 2 or 4. This is generally true of the plants we have studied and leads to a systematic departure from linearity of the relationship of $P.I.$ to time. No attempt has been made to correct for this error. From plastochron 5 on, the leaves seem to appear in a consistent (2 + 3) phyllotactic sequence, and there seem to be no systematic departures from equality of successive plastochrons correlated with the morphology of the shoot. In short, $P.I.$ should be a more satisfactory index of development after plastochron 5 than before. After about $P.I.$ 13 there is a decrease in slope of the curve. This is undoubtedly to be explained by root-binding of the plant, since in several similar plants the original rate was restored by transferring the plant to a larger pot. A straight line has been fitted by least squares to these data up to about $P.I.$ 13 and the

standard error of estimated $P.I.$ calculated to be 0.018. The slope of the fitted line, r/p , is 0.47 day⁻¹, and the average duration of the plastochron, p/r , is therefore 2.12 days. The standard error, 0.018 plastochron, is therefore equivalent to slightly less than one hour. For other plants standard errors of estimate of 0.15 or less have been calculated, and it is clear that under our conditions the plastochron index serves to specify the developmental status of a shoot with an error of only a few hours.

THE PLASTOCHRON INDEX OF A LEAF.—For studies of leaf development, it is convenient to alter (1) to provide a common scale of plastochrons for any leaf on a shoot. This can be done for any leaf by subtracting its serial number from the plastochron index of the plant on which it is borne. The resulting leaf plastochron index for a given leaf, whose serial number is a , is

$$(L.P.I.)_a = (P.I.) - a = n - a + \frac{\ln L_n - \ln 10}{\ln L_n - \ln L_{n+1}} \quad (6)$$

On this scale, a leaf exactly 10 mm. long will have a plastochron age of zero, a leaf shorter than 10 mm. will have a negative plastochron age, and one longer than 10 mm. will have a positive value of the index. $L.P.I.$ values have been calculated for each leaf listed in table 1 on each of the six days. Note that on a given date the index increases by one for each successive leaf, counting from the apex toward the base. For a given leaf, it increases approximately linearly with time. This is true even for leaves such as numbers 3 and 4 which have ceased elongating, and from whose length alone no index of age could be obtained.

APPLICATION OF THE PLASTOCHRON INDEX OF THE SHOOT.—The plastochron index as defined by (1) applies to the shoot, and should be useful in developmental studies in which interest centers on characteristics of the shoot or entire plant, such as

total fresh or dry weight, content of a biochemical constituent, rate of a metabolic process, etc. While we have no data to illustrate this application, some suggestions might be made. It is usual in such studies to plant a crop of the plant in question in the field or greenhouse, and to dig samples of plants for analysis at weekly or other intervals during the growing period. Because of variability of the plants in their response to environmental fluctuations, fairly large samples are usually used. Average values are then plotted against time. We would propose that in such a study the plastochron index of each plant be found, and that analyses be made on individual plants. The analytical results for each plant could then be plotted against its *P.I.* Plotting *P.I.* against time would provide the information necessary to interpret the *P.I.* scale in temporal terms. It is convenient, though not necessary, that the *P.I.* be a linear function of time. It might be desirable in some cases to plant at frequent intervals until a wide selection of plants in various stages of development are at hand, and at each sampling time to take plants of as wide a range of stages as feasible. This procedure would have the effect of confounding the environmental variability, and should give more precise information with an economy of plants and analytical work. Some limitations of this application of the plastochron index are discussed below.

In experimental studies of the effect of various regimes of light and temperature conditions, nutritional treatments, etc., the rate of progression, r/p , of the plastochron index might be determined for a few plants under each set of conditions. This sort of study would have interest in itself in many cases. Other plants grown under the same sets of conditions could then be taken at various plastochron ages, for analysis. If dry weights, for instance, were determined, rates of change in dry weight per plastochron could be evaluated from a plot of dry weight against *P.I.* These rates multiplied by r/p would yield rates of change of dry weight with time.

The plastochron index should also serve for studies of the development of the shoot apex, such as those of Abbe and Phinney (1951), in which a progressive increase in mass of the apical meristem of *Zea* during the growing season was demonstrated, and Zimmermann (1928) who demonstrated cyclical changes in area of the meristem correlated with leaf initiation in *Hypericum*. Much would be gained by determining the precise plastochron age of each apex, rather than simply grouping them into classes corresponding with integral values of *P.I.*, as Abbe and Phinney have done, or judging the plastochron phase subjectively as in Zimmermann's work. A reference length of the order of 100 μ , which could be measured microscopically, might be suitable in this application.

APPLICATION OF THE LEAF PLASTOCHRON INDEX.—For studies of leaf development, the *L.P.I.* is

applicable. This may be illustrated in a simple manner by replotted the leaf lengths of fig. 1 against the *L.P.I.* for each leaf (fig. 7). It is apparent that the growth curves for leaves 3, 5, 7, 9, and 11 are very similar in early stages, and that they differ in the plastochron age at which the relative rate of elongation begins to decrease, as well as in the final lengths attained. A more useful application of the leaf index is reported by Michelini (1955). Fresh weight, chlorophyll content and rate of oxygen uptake were determined for *Xanthium* leaves at various stages of development. The great scatter which resulted from plotting these determinations against days from planting was dramatically reduced by plotting the same values against *L.P.I.* Maksymowych (1956) has also applied the leaf plastochron index to a study of histogenesis of *Xanthium* leaves. A much clearer picture of the temporal relationships of such processes as marginal meristem activity and mesophyll development can be had from this study than from the classical descriptions of leaf development.

DISCUSSION.—Many plant physiologists and morphologists have selected plant material for study or experiment on the basis of measurements or morphological criteria of development. For instance, *Avena* coleoptiles of specified length are used in the standard tests for auxin, and one frequently finds statements that only plants with a specified number of leaves were used for given experiments. In effect, a developmental index is frequently used to provide more or less standardized plant material. We cannot attempt here to review these procedures in any comprehensive way, but we shall discuss a few examples which have come to our attention.

Xanthium is widely used in photoperiodic investigations, and the procedures which have been used for standardizing this material are of interest here. Whole plants which have been grown for a speci-

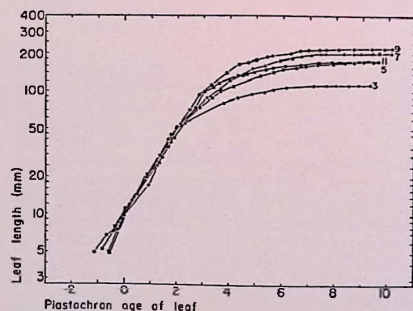


Fig. 7. Lengths of leaves 3, 5, 7, 9, and 11 plotted logarithmically against the leaf plastochron index of each. Same data as fig. 1.

fied time under noninductive conditions have been used in most of the work, and it is sometimes specified that only plants with a given number of expanded leaves have been used. In some cases plants have been defoliated. Parker et al. (1946), for instance, defoliated their plants to a single leaf, which was said to be the youngest "fully-expanded" leaf, and was usually at node 6 or 7. They presumably left an unspecified number of young leaf primordia at the apex. Similar methods of preparing *Xanthium* plants have been used by Bonner and Thurlow (1949), Liverman and Bonner (1953), and others. Khudairi and Hamner (1951) have made a study of the effectiveness of leaves of various developmental stages in photoperiodic induction, using leaf areas and to some extent node numbers as indications of the developmental status of the leaves. Salisbury (1955a, b) has perhaps made the most careful use of such methods of standardization in *Xanthium*. He refers to successive leaves longer than 10 mm. at a given time as the No. 1 leaf, No. 2 leaf, etc., counting from apex to base. These numbers correspond, not to the serial numbers (n) which we have assigned to the leaves, but to values of the leaf plastochron index, rounded off to the nearest integer. His fig. 1 is then a plot of leaf length against *L.P.I.* such as our fig. 7. We have calculated from the arbitrary lengths of his No. 1 leaf and No. 2 leaf, 18 and 42 mm. respectively, that the relative plastochron rate, p , is 0.85, in reasonable agreement with our data, and also that his reference leaf length, that is, the length corresponding to $L.P.I. = 0$, is about 7.7 mm. A closer comparison of his methods with ours is not feasible since he has presumably measured leaf blade lengths whereas we have measured total leaf lengths. Incidentally, Salisbury's system of floral stages constitutes a good developmental index for the inflorescence, since he shows (fig. 3) that floral stage increases linearly with time. We feel that the use of the leaf plastochron index to specify leaf age would be appropriate in studies such as these of Khudairi and Hamner, and Salisbury, and would allow more precise standardization.

In his studies of leaf abscission and of xylem regeneration after wounding in *Coleus*, Jacobs (1952) has similarly designated leaf-pairs of increasing age with successive numbers. Similarly, his leaf numbers are integral values of the leaf plastochron index. He states that plants were selected in which leaf-pair no. 1 is 30–50 mm. long and leaf-pair no. 2 is 70–100 mm. long. These values yield a value of the relative plastochron rate, p , of about 0.76 and a reference length of about 18 mm. In preliminary data on the growth of *Coleus* which we have collected, it appears appropriate to use a reference length of 20 mm., since the young leaves are not accessible until about 10–12 mm. long. We suggest that the *L.P.I.* would be quite appropriate for studies of the sort

which Jacobs envisages, and would lead to closer correlations with leaf development.

Higgins (1952) has proposed a developmental index for "phenological" observations of cultivated peas (*Pisum*) which has much in common with the plastochron index here described. He proposed counting the number of "fully-developed nodes" on a pea plant, and adding to this integer a decimal fraction representing the stage of development reached by the "terminal bud." The integral part of his index corresponds to the integral part of the plastochron index as here defined. He provides a series of ten drawings and ten descriptive statements to define tenths of a plastochron. We have been told by Dr. F. W. Went that in his experience Higgins' index is not linear with time for fractional parts of a plastochron. We have therefore taken a limited series of photographs of a developing pea shoot with an automatic camera, from which it can be concluded that Higgins' value of 0.5 plastochron corresponds to an actual value of about 0.3. Higgins' index would be a very convenient one provided that accurate descriptive criteria of fractions of a plastochron could be found. In the case of our photographs, however, it is evident that no striking morphological criteria are at hand except during the earlier part of the plastochron as Higgins has defined it. Rather, the changes are quantitative and measurements are, of course, appropriate. The pea leaf, however, is awkward to measure, since it is terminated by a tendril, and in any case is enclosed tightly by the stipules in early stages. From preliminary observations, it seems appropriate in the pea plant to use internode lengths in calculation of a plastochron index. We would suggest assigning to each internode the same serial number as that of the leaf subtending it, and using internode lengths in formula (1) in place of leaf lengths, taking 20 mm. as the reference internode length.

It may be noted that the names orange pekoe, pekoe, souchong, etc., which are used in grading of tea (Hill, 1937), constitute a crude leaf plastochron index.

From the discussion above it may be supposed that the plastochron index as formulated for *Xanthium* would be applicable in studying vegetative shoot growth in a variety of herbaceous dicotyledons, with modifications depending on the morphology of the plant in question. The index fails in *Xanthium* when flowering is induced, since leaf initiation is halted, and this same limitation may apply to many plants. In studying vegetative development, then, it would be desirable, for this and other reasons, to choose plants and growing conditions which will provide strictly vegetative growth. For the study of flowering phenomena, the indices developed by Erickson (1947) for *Lilium* flowers and by Salisbury (1955) and others for *Xanthium* inflorescences are suggestive of ways in which developmental indices may be derived.

In some groups of plants, difficulty would perhaps be encountered in finding an appropriate basis for a plastochron index. In grasses, for instance, young leaves and internodes are enclosed tightly in the sheaths of more mature leaves. On dissection, the young leaves could be measured and a plastochron index calculated for each dissected plant. The difficulty, however, of establishing the relation between this plastochron index and time is one for which we do not see a solution at present. Difficulties might also be encountered in plants with marked seasonal growth. In several tree species which we have observed casually the winter bud contains several leaf primordia of nearly the same length. They expand rapidly and nearly simultaneously after the buds open, and may or may not be followed by later leaves, which follow a plastochronic sequence like that in *Xanthium*. Whether a useful developmental index could be formulated for this process, and the many other growth processes exhibited by plants, would depend in each case on a careful working-out of the growth pattern in time, and some ingenuity in selecting appropriate measurements or criteria which can be related to time in a precise way.

Developmental indices could no doubt be used to advantage in the study of other organisms than the higher plants. Stages of normal development have been defined with greater or lesser formality for many organisms. In vertebrate embryology, for instance, the series of normal stages defined by Harrison and others for a number of amphibians (Hamburger, 1942; Rugh, 1948) and by Hamburger and Hamilton (1951) for the chick embryo are in effect developmental indices, and as we understand, have great utility in experimental em-

bryology. The available data show, however, that the relationship of stage to time is not simple, perhaps because the numbered stages have been chosen primarily for convenience of definition in morphological terms. Furthermore, the possibility of numerical interpolation between the stages does not appear to have been considered seriously. We would suggest that in many organisms numerical stage series, or developmental indices, preferably defined so as to be linear with time, would have great advantages, not only for the standardization and evaluation of experimental material, but also for the calculation of rates of many processes involved in development.

SUMMARY

An index which specifies precisely the developmental status of a vegetative shoot is derived, with particular reference to *Xanthium*. This *plastochron index* has the formula

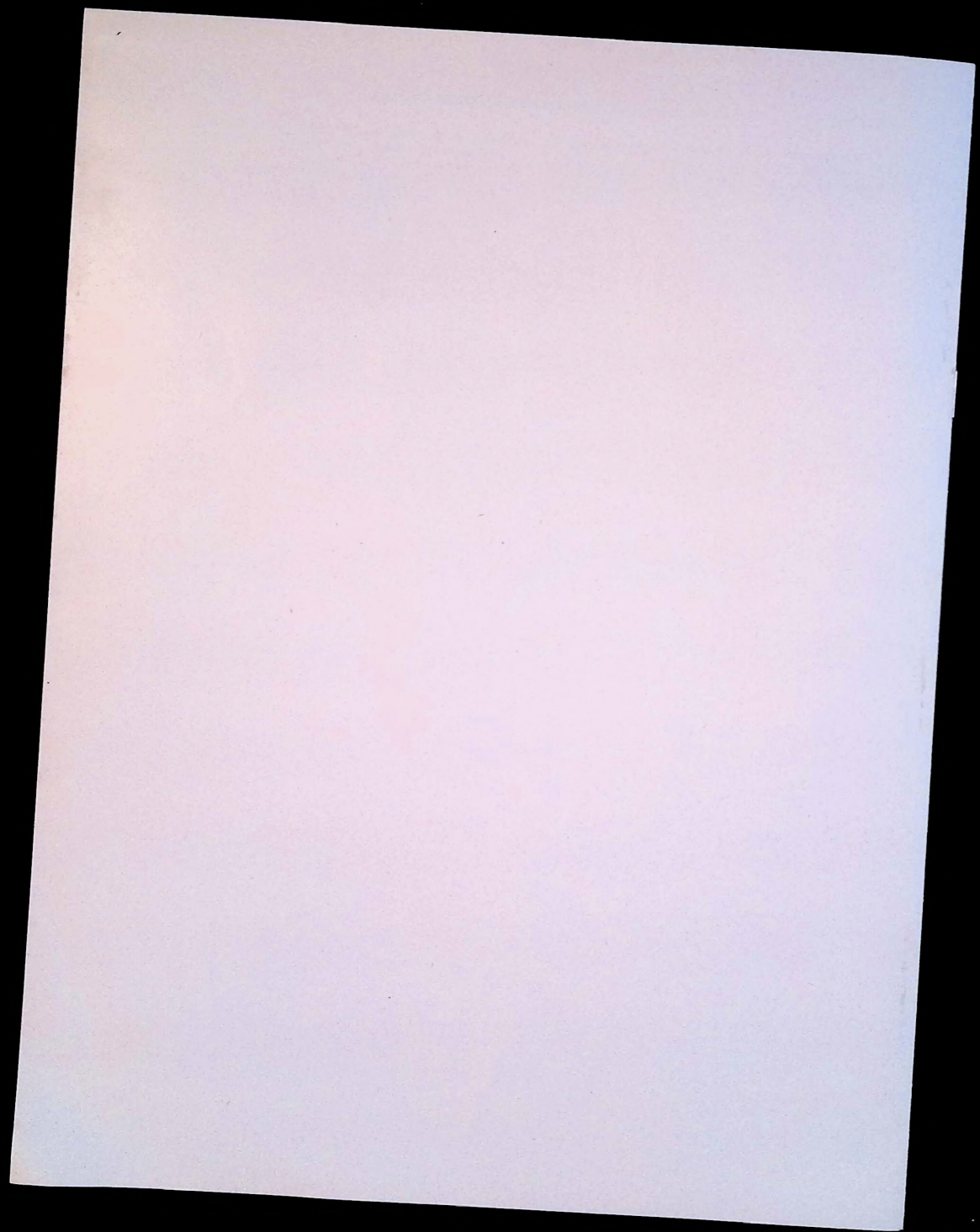
$$P.I. = n + \frac{\log L_n - \log 10}{\log L_n - \log L_{n+1}}$$

where n is the serial number of the leaf just longer than an arbitrary reference length, 10 mm., and $\log L_n$ is the logarithm of the length of leaf n . *P.I.* is shown to be linearly related to time during vegetative growth, and can therefore be used as a substitute time axis in many studies. The use of the plastochron index in studies of shoot development, and of the similar leaf plastochron index (*L.P.I.*) in studies of leaf development is advocated.

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LITERATURE CITED

- ABBE, E. C., AND B. O. PHINNEY. 1951. The growth of the shoot apex in maize: external features. *Amer. Jour. Bot.* 38: 737-744.
- ASKENASY, E. 1880. Über eine neue Methode, um die Verteilung der Wachstumsintensität in wachsenden Theilen zu bestimmen. *Verh. Naturh.-medic. Ver. Heidelberg* 2: 70-153.
- BLACKMAN, V. H. 1919. The compound interest law and plant growth. *Ann. Bot.* 33: 353-360.
- BONNER, J., AND J. THURLOW. 1949. Inhibition of photoperiodic induction in *Xanthium* by applied auxin. *Bot. Gaz.* 110: 613-624.
- ERICKSON, R. O. 1947. Respiration of developing anthers. *Nature* 159: 275.
- . 1948. Cytological and growth correlations in the flower bud and anther of *Lilium longiflorum*. *Amer. Jour. Bot.* 35: 729-739.
- ESAU, K. 1953. *Plant anatomy*. John Wiley and Sons, Inc. New York.
- HAMBURGER, V. 1942. *A manual of experimental embryology*. Univ. of Chicago Press. Chicago.
- , AND H. L. HAMILTON. 1951. A series of normal stages in the development of the chick embryo. *Jour. Morph.* 88: 49-92.
- HIGGINS, J. J. 1952. Instructions for making phenological observations of garden peas. *Johns Hopkins Univ. Publ. Climatol.* 5(2): 1-11.
- HILL, A. F. 1937. *Economic botany*. McGraw-Hill Book Co. New York.
- JACOBS, W. P. 1952. The role of auxin in differentiation of xylem around a wound. *Amer. Jour. Bot.* 39: 301-309.
- KHUDAIRI, A.-K., AND K. C. HAMNER. 1954. The relative sensitivity of *Xanthium* leaves of different ages to photoperiodic induction. *Plant Physiol.* 29: 251-257.
- LIVERMAN, J. L., AND J. BONNER. 1953. Biochemistry of the photoperiodic response: the high-intensity-light reaction. *Bot. Gaz.* 115: 121-128.
- MAKSYMOWYCH, R. 1956. Development of lamina in *Xanthium italicum* Moretti represented by the plastochron index. (M.S. thesis). Univ. Pennsylvania.
- MICHELINI, F. J. 1955. The use of the plastochron index in studies of morphological and physiological development in *Xanthium italicum* Moretti. (Ph.D. dissertation). Univ. Pennsylvania.
- OCUR, M., R. O. ERICKSON, G. U. ROSEN, K. B. SAX, AND C. HOLDEN. 1951. Nucleic acids in relation to cell division in *Lilium longiflorum*. *Exp. Cell Res.* 2: 187-203.
- PAKER, M. W., S. B. HENDRICKS, H. A. BORTHWICK, AND N. J. SCULLY. 1946. Action spectrum for the photoperiodic control of floral initiation of short-day plants. *Bot. Gaz.* 108: 1-26.
- RUGH, R. 1948. *Experimental embryology*. Burgess Publishing Co. Minneapolis.
- SALISBURY, F. B. 1955a. Kinetic studies on the physiology of flowering. (Ph.D. dissertation). California Inst. Tech.
- . 1955b. The dual role of auxin in flowering. *Plant Physiol.* 30: 327-334.
- ZIMMERMANN, W. A. 1928. Histologische Studien am Vegetationspunkt von *Hypericum uratum*. *Jahrb. Wiss. Bot.* 68: 289-344.



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*From the files of
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STAPHYLOCOCCUS AUREUS ANTIGENS IN HYPERSENSITIVITY
REACTIONS AND EXPERIMENTAL ARTERIAL SENSITIZATION

SHELDON G. COHEN, M.D.,* DANIEL S. DZURY, M.S.,** AND FRANCIS J.
MICHELINI, Ph.D.,*** WILKES-BARRE, PA.

WITH THE TECHNICAL ASSISTANCE OF VINCENT A. J. DRAPIEWSKI****

INTRODUCTION

INVESTIGATION into the subject of bacterial sensitivity has been hampered by the physical state of the bacterial cell itself participating in in vivo antigen-antibody reactions. It is always difficult to evaluate the role of an intact cell as a true antigen in studying hypersensitivity phenomena, as its particulate nature and cellular function might suggest a biologic effect apart from a suspected role in immunologic reactions. Then, too, biochemical laboratory procedures designed to fractionate the living cell into protein, carbohydrate, and lipid components entail the risk of creating new compounds that might result from the splitting and/or rearrangement of the labile, complex, molecular aggregates that may have been characteristic of intracellular structure.¹ The report of Dworetzky and co-workers² has demonstrated the physiologic and immunologic action of *Staphylococcus aureus* preparations obtained by grinding live microorganisms in a ball mill, and our studies³ have demonstrated the retained antigenicity of *Staphylococcus aureus* cytoplasm recovered from the disruption of live bacterial cells when exposed to ultrasonic vibrations. This availability of antigenic bacterial cytoplasm has furnished a convenient tool for the further study and consideration of bacterial hypersensitivity for in a soluble state biologically active *Staphylococcus aureus* bacterial preparations may lend themselves to participation in such attempted in vivo antigen-antibody reactions as anaphylactic shock, Arthus cutaneous reactions, and experimentally induced lesions of vascular sensitization in experimental animals.

From the Department of Biology, Wilkes College.

Presented in part at the thirteenth annual meeting of the American Academy of Allergy, Los Angeles, California, Feb. 4-6, 1957.

This investigation was supported (in part) by a research grant (No. H-2412) from the National Heart Institute, Public Health Service.

Soluble bacterial cell protoplasm employing ultrasonic vibrations was prepared under the direction of Dr. Sam Beiser, Department of Microbiology, Columbia University, College of Physicians and Surgeons, New York.

Quantities of *Staphylococcus aureus* live bacterial organisms in a frozen and dried state and staphylococcus toxoid were kindly supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, through the courtesy of Dr. H. D. Piersma.

Received for publication April 15, 1957.

*Associate Research Professor of Biology.

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EXPERIMENTAL PROCEDURES AND RESULTS

Antigens.—All bacterial antigens used for immunization and challenging procedures were derived from broth cultures of the same strain of *Staphylococcus aureus*, Wood 46.

1. **Vaccine:** Saline suspension of phenol killed whole bacterial cells.

2. **Undenatured cellular protoplasm:** Prepared by subjecting saline suspensions of whole live bacterial cells to the effects of ultrasonic waves at 17,000 cycles per second. Bacterial debris consisting chiefly of cell walls was separated from the soluble cellular contents by centrifugation and Seitz filtration. The resultant sterile filtrate contained 1 mg. nitrogen per milliliter.

3. **Toxoid:** A culture filtrate of *Staphylococcus aureus* treated by pepsin digestion and containing 10,000 dermonecrotizing units per milliliter.

Experimental Animals.—Male rabbits varying between 2.5 and 3.5 Kg. in weight were used throughout in active immunization procedures for the production of antisera and for all passive sensitization and challenge experiments.

Antisera.

1. **Antibacterial sera:** This was prepared by immunizing rabbits with intravenous and intradermal injections of vaccine for a total dose of 21 billion bacterial organisms administered in divided doses over a twenty-one-day period. Seven days after the last injection the rabbits were bled by cardiac puncture; the sera were pooled and sterilized by Seitz filtration. The antiserum was capable of agglutinating *Staphylococcus aureus* bacterial cells in in vitro macroscopic tests in a serum titer of 1:2,560 and formed interfacial precipitate when overlaid in test tubes with saline dilutions of *Staphylococcus aureus* soluble cytoplasm and toxoid.

2. **Antitoxin:** This was prepared by immunizing rabbits with intramuscular injections of toxoid adsorbed on aluminum hydroxide cream adjuvant at multiple sites for a total dose of 100,000 dermonecrotizing units. Twenty-one days later the animals were bled by cardiac puncture; the sera were pooled and sterilized by Seitz filtration. This antiserum was capable of agglutinating *Staphylococcus aureus* bacterial cells in in vitro macroscopic tests in a serum titer of 1:80 and formed interfacial precipitate when overlaid in test tubes with saline dilutions of *Staphylococcus aureus* soluble cytoplasm and toxoid.

Anaphylaxis.—Anaphylaxis was uniformly produced in normal rabbits following passive sensitization and challenge experiments with *Staphylococcus aureus* antisera-antigen systems. Passive sensitization was accomplished by the intravenous administration of 5 ml. of homologous species antisera capable of agglutinating *Staphylococcus aureus* bacterial cells in a serum titer of 1:2,560.

Thirty minutes later they were challenged with a soluble preparation of *Staphylococcus aureus* cellular protoplasm. Within 2 to 3 minutes after receiving the minimal critical dose of 0.25 mg. N, typical signs and symptoms of fatal anaphylactic shock appeared with the resultant gross pathologic finding of right heart dilatation. Abnormal signs and symptoms were completely absent in the control series of animals receiving either the 5 ml. of antiserum or 0.25 mg. N of soluble bacterial cell protoplasm alone.

Cutaneous Reactions.—The intradermal injection of saline dilutions of *Staphylococcus aureus* soluble cell protoplasm preparations in normal rabbits resulted in the development of cutaneous lesions of erythema, edema, and induration over a twenty-four to forty-eight-hour period at the site of injection and usually progressed to resemble the dermonecrotic lesions observed in rabbits following the intradermal injection of *Staphylococcus aureus* culture filtrate or toxoid. The development of these skin lesions following antigen injections alone prevented the study and evaluation of the ability of *Staphylococcus aureus* antigen-antibody systems to participate in in vivo cutaneous hypersensitivity reactions of the Arthus reaction type in sensitized rabbits prepared for challenge by the intradermal route. Cutaneous reactions failed to develop in rabbits in whom local skin sites were prepared by intracutaneous injections of antibacterial agglutinating antiserum or precipitating antitoxin and subsequently challenged with the intravenous administration of soluble bacterial cell protoplasm and toxoid, respectively.

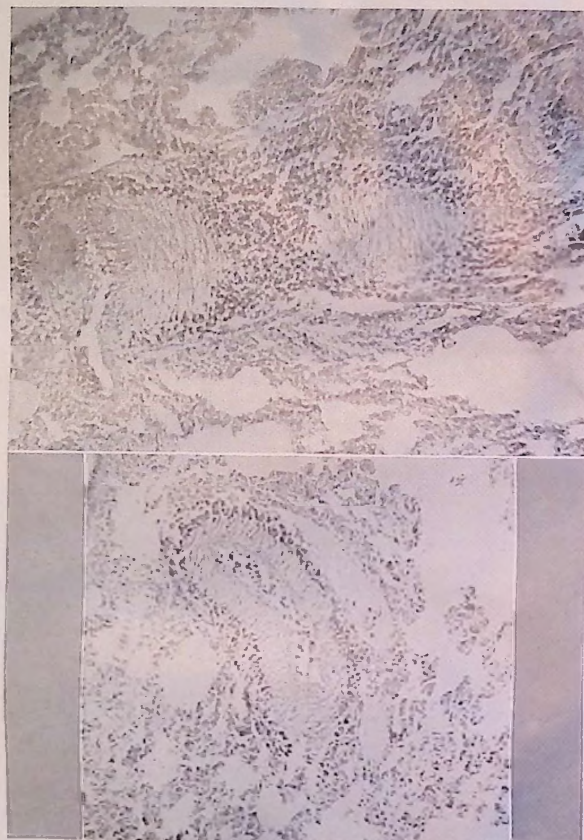
Arteritis.—The techniques previously described, favorable for the production of lesions of pulmonary periarteritis and panarteritis with horse serum-antihorse serum⁴ and streptococcus toxin-antitoxin⁵ systems, were extended to evaluate potential vascular sensitizing properties of *Staphylococcus aureus* antigen-antibody systems. In this procedure normal animals are passively sensitized with the intravenous administration of a high-titer antiserum. After an incubation period of thirty minutes the subsequent intravenous challenge

TABLE I. THE NUMBER OF RABBITS DEVELOPING LESIONS OF PULMONARY PERIARTERITIS AND PANARTERITIS FOLLOWING PASSIVE SENSITIZATION WITH HOMOLOGOUS SPECIES RABBIT AGGLUTINATING ANTISERA VERSUS STAPHYLOCOCCUS AUREUS BACTERIAL CELLS OR ANTITOXIN AND SUBSEQUENTLY CHALLENGED WITH SOLUBLE CELL PROTOPLASM OR TOXOID OF THE SAME BACTERIAL SPECIES

NO. OF ANIMALS	SENSITIZING ANTISERUM VERSUS STAPHYLOCOCCUS AUREUS	CHALLENGING ANTIGEN STAPHYLOCOCCUS AUREUS	NO. OF ANIMALS WITH LESIONS
6	5 ml. antiserum for whole cell	Cell protoplasm 0.25 mg. N	5
6	5 ml. antiserum for whole cell	—	0
6	—	Cell protoplasm 0.25 mg. N	0
5	5 ml. antiserum for whole cell	Toxoid 5,000 dermonecrotizing units	4
5	5 ml. antitoxin	Cell protoplasm 0.25 mg. N	4
5	5 ml. antitoxin	Toxoid 5,000 dermonecrotizing units	4
6	—	Toxoid 5,000 dermonecrotizing units	0

The control series of animals received only the antisera or preparations of the bacterial antigens.

A.

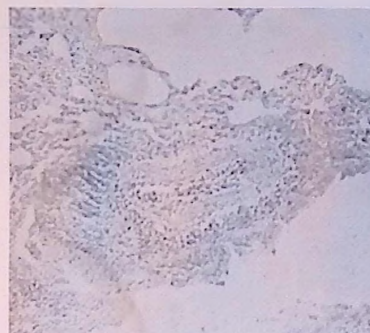


B.

Fig. 1.—Sections of lung tissue from rabbits that had been passively sensitized with homologous species *Staphylococcus aureus* antisera and subsequently challenged with the intravenous administration of subanaphylactic doses of soluble *Staphylococcus aureus* antigens. The involved arteries and arterioles show marked perivascular and adventitial infiltration of eosinophils and some polymorphonuclear leukocytes and, to a lesser extent, of a few lymphocytes. Some intimal reaction can be seen in A and C. Eosinophils can be seen penetrating vessels seen in B and C, and early thrombus formation has occurred within the lumen of one vessel seen in B. High-power magnifications of sections E and F demonstrate the eosinophilic cellular perivascular and adventitial infiltrates.

(Fig. 1, C to G on opposite page.)

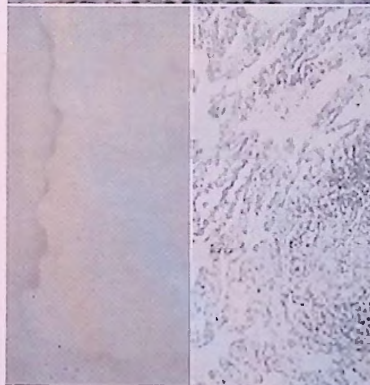
C.



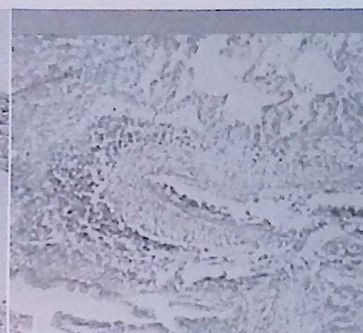
E.



G.



D.



F.

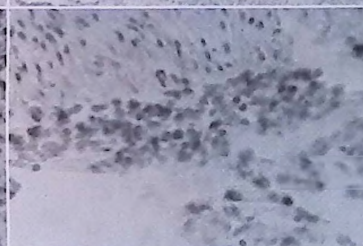


Fig. 1.—C to G. (For legend, see opposite page.)

of these animals with the soluble specific antigen is started. A total dosage of antigen previously determined to be the minimal critical dose capable of producing fatal anaphylactic shock is divided into four fractional dosages, each given at fifteen-minute intervals in graded increasing concentrations over the period of one hour in an attempt to effect an asymptomatic subacute anaphylactic reaction. Twenty-four hours later the animals are sacrificed by air embolism; sections are taken from the internal organs, prepared, and stained for histologic study.

The *Staphylococcus aureus* heterologous species rabbit antisera used for passive sensitization consisted of 5 ml. of either an antiserum for the whole cell with a bacterial agglutinating titer of 1:2,560 or a precipitating antitoxin. The *Staphylococcus aureus* antigens used in challenging the passively sensitized rabbits consisted of either: (1) soluble bacterial cell protoplasm in divided doses of 0.025, 0.05, 0.75, and 0.1 mg. N, or (2) toxoid in divided doses of 500, 1,000, 1,500 and 2,000 dermonecrotizing units. The results are summarized in Table I.

Passive sensitization with antisera for the whole bacterial cell: When challenged with soluble cell protoplasm, five out of six rabbits showed pulmonary vascular lesions. When the animals were challenged with toxoid, pulmonary vascular lesions were demonstrated in four out of five rabbits.

Passive sensitization with antitoxin: When challenged with toxoid, four out of five rabbits showed pulmonary vascular lesions. When the challenge was made with soluble cell protoplasm, pulmonary vascular lesions were demonstrated in four out of five animals.

Lesions of the pulmonary vasculature could not be found in six rabbits receiving only antiserum for the whole cell, five rabbits receiving antitoxin, six rabbits receiving only soluble cell protoplasm, and six rabbits receiving toxoid alone.

In those series of animals where lesions were demonstrated following passive sensitization and challenge experiments, the pulmonary vasculature was found to be chiefly involved (Fig. 1). The small pulmonary arteries and pulmonary arterioles showed marked perivascular and adventitial infiltration of eosinophils and, to a lesser extent of polymorphonuclear leukocytes and some lymphocytes. Eosinophils were also found infiltrating the intima and media of the small arteries and arterioles, and in several instances collections of eosinophils and early thrombus formation were found within the lumina of these vessels. In several sections varying degrees of hyperplasia of the endothelial cells of the intima, cloudy swelling of the muscle fibers with hydropic degeneration of the media, and some adventitial edema were found. Scattered and light interstitial infiltration with some eosinophils was seen occasionally within some alveolar walls.

DISCUSSION

Typical symptoms and gross pathologic findings of anaphylactic shock in rabbits were uniformly elicited in animals passively sensitized by the intravenous

administration of homologous species *Staphylococcus aureus* antibacterial agglutinating sera and subsequently challenged with the soluble cellular protoplasmic contents of the same bacterial strain. Bacterial cells have previously been shown capable of causing anaphylactoid reactions in sensitized animals, but the role of the cells as antigen in these instances has been difficult to evaluate because of the toxicity of the bacterial preparations, per se, and because of their particulate nature. Thus, it has not been possible to rule out their role as intact particles in possible thrombus formation and/or embolization.⁶ However, the reduction of cells to undenatured cellular antigen by the gentle but effective disrupting action of ultrasonic waves on the cell wall and the employment of this soluble material as the challenging antigen would tend to avoid such complicating factors and offer evidence to suggest a true sensitizing role for *Staphylococcus aureus* cytoplasm in such in vivo reactions of hypersensitivity as anaphylaxis. Little need be said regarding the value of the established technique of passive sensitization and challenge in allowing for the controlled evaluation of in vivo effects of the action of either antibacterial serum or the *Staphylococcus aureus* antigen. This further lends itself to the establishment of a minimal critical dose of challenging antigen prerequisite for anaphylaxis. In this regard, its value in circumventing the interfering factor of possible anaphylactoid reactions in nonsensitized animals resulting from toxic effects of larger doses of *Staphylococcus aureus* bacterial antigen is evident.

It was impossible to evaluate any potential for localized skin-sensitizing effects of intracutaneously administered soluble bacterial cell protoplasm because of the local cutaneous reactions of erythema, edema, induration, and progression to necrosis that resulted from the local injection of this material in dosages considered prerequisite for interreaction with specific antibody-containing homologous species serum for the production of local Arthus reactions. Kahn⁷ has noted similar inflammatory type "flare-up" reactions in rabbits injected intradermally with killed suspensions of staphylococcus and attributed these to the natural immunity possessed by this animal to these microorganisms. However, the results of agar gel diffusion plates in an attempt to resolve *Staphylococcus aureus* cytoplasm into constituent antigens would suggest that the dermonecrotizing effects observed may be partially due to the actual exotoxin content demonstrated within the bacterial cell itself in the particular strain of this microorganism studied and reported here.⁸

Previous studies^{4, 5, 8-10} employing the technique of passive sensitization and challenge have demonstrated that pulmonary lesions of periarteritis and panarteritis in the rabbit arise as a result of in vivo antibody-antigen reaction and probably as a tissue manifestation of subacute anaphylaxis. The results of the experimental procedure concerned with *Staphylococcus aureus* antibody-antigen systems reported here would tend to offer further evidence in this regard. These lesions may arise as a result of hypersensitivity reactions involving either the cellular cytoplasm and/or the exotoxin of *Staphylococcus aureus* and do not differ significantly in histologic detail from those resulting from the action of other protein antigen-antibody systems, such as horse serum-antihorse

serum. Their development, therefore, probably depends upon a tissue response to reactions of hypersensitivity rather than to any particular specific characteristic of the nature of the bacterial antigen or antiserum employed.

In vitro cross reactions in macroscopic agglutination and precipitin tests and precipitin band reactions of identity in agar gel diffusion plates have been observed between various *Staphylococcus aureus* cellular protoplasmic and toxoid antigens when tested against their respective antisera.³ These probably arise as a result of each sharing some immunologically similar or identical component antigens. The fact that tissue lesions of vascular sensitization can arise in rabbits after challenge with either staphylococcus cellular protoplasm or toxoid, regardless of whether the animal has been passively sensitized to the bacterial antisera or to the antitoxin, would point out the possibility of similar cross reactions occurring in vivo, as well as in in vitro test procedures. Ehrlich¹¹ has observed the development of primary mesenchymal reactions in rabbits following the intravenous injection of whole bacteria. However, the control studies utilizing only preparations of cell protoplasm, toxoid, antibacterial serum, or antitoxin suggest that lesions of pulmonary periarteritis and panarteritis described here arise as a result of tissue response to *Staphylococcus aureus* antibody-antigen reactions apart from any other tissue effect, toxic or otherwise, that may possibly be exerted by *Staphylococcus aureus* antigens or the presence of their antisera.

SUMMARY

1. The sensitizing properties of *Staphylococcus aureus* (Wood 46 strain) were studied, utilizing soluble cell protoplasm obtained by exposing live bacterial cells to the disrupting effects of ultrasonic vibrations and pepsin-digested culture filtrate (toxoid) as antigens.

2. In rabbits passively sensitized with high-titer bacterial agglutinating antisera, fatal anaphylaxis could be uniformly elicited by subsequent intravenous challenge with a soluble preparation of bacterial cytoplasm.

3. When the previously determined critical anaphylactic dose of soluble bacterial cellular protoplasm was administered intravenously in fractional dosages over a sixty-minute time period to rabbits passively sensitized with the same high-titer bacterial agglutinating antiserum, lesions of pulmonary periarteritis and panarteritis were demonstrated twenty-four hours later.

4. Lesions of pulmonary panarteritis and periarteritis were also observed when similar techniques were employed in rabbits passively sensitized with high-titer bacterial agglutinating antiserum and challenged with toxoid and in rabbits passively sensitized with antitoxin and challenged with either toxoid or soluble bacterial cellular protoplasm.

5. Neither anaphylactoid reactions nor the development of lesions of pulmonary periarteritis or panarteritis were observed in normal rabbits receiving only the same bacterial agglutinating antisera, antitoxin, soluble bacterial protoplasm, or toxoid.

6. The intracutaneous injection of preparations of soluble bacterial cellular protoplasm in normal nonsensitized rabbits resulted in the development of cutaneous lesions of erythema, edema, infiltration, and necrosis. This resembled the dermonecrotizing action of *Staphylococcus aureus* toxin and may have been due to the antigenic exotoxin components previously demonstrated within *Staphylococcus aureus* bacterial cells.

7. The sensitizing properties of the cytoplasm and toxin of *Staphylococcus aureus*, their antigenic relationships, and their ability to participate in such in vivo reactions of hypersensitivity in the rabbit as anaphylaxis and the development of tissue manifestations of vascular sensitization have been demonstrated.

REFERENCES

1. Boyd, W. C.: *Fundamentals of Immunology*, ed. 3, New York, 1956, Interscience Publishers, Inc., p. 211.
2. Dworetzky, M., Baldwin, H. S., and Smart, K. M.: Biologic Effects of Materials Derived From *Staphylococcus*: Further Studies, *J. ALLERGY* 27: 39, 1956.
3. Cohen, S. G., Dzury, D. S., and Michelini, F. J.: Immune Responses to *Staphylococcus Aureus* Antigens, (To be published.)
4. Cohen, S. G., Mayer, L. D., and Criepp, L. H.: The Experimental Production of Arteritis by Passive Sensitization, *J. Immunol.* 66: 487, 1951.
5. Cohen, S. G., Mokyele, W. E., and Drapiewski, J. F.: The Etiological Role of Streptococcus Toxin-Antitoxin Systems in the Experimental Production of Arterial Sensitization, *J. ALLERGY* 24: 411, 1953.
6. Chase, M. W., in Dubos, R. J.: *Bacterial and Mycotic Infections of Man*, ed. 2, Philadelphia, 1952, J. B. Lippincott Company, p. 177.
7. Kahn, R. L.: Tissue Response in Immunity, *Ann. New York Acad. Sc.* 59: 281, 1955.
8. Cohen, S. G., and Moses, C.: Effect of Cortisone on Experimental Production of Arteritis by Passive Sensitization, *J. Lab. & Clin. Med.* 57: 764, 1951.
9. Cohen, S. G., Walsh, J. W., and Dzury, D. S.: The Effect of Biologic and Chemical Agents on the Experimental Production of Arteritis by Passive Sensitization. II. Nitrogen Mustard, *J. Immunol.* 73: 100, 1954.
10. Cohen, S. G., Dzury, D. S., and Walsh, J. W.: Experimental Arterial Sensitization. Studies on the Specificity of "Shock Tissue," *J. ALLERGY* 27: 461, 1956.
11. Ehrlich, W. E.: The Significance of Tissue Reactions Caused by Antigens, *J.A.M.A.* 135: 94, 1947.



Friday, February 26, 1960

WIZARD OF CHAWS

Dr. Michelini Retains Title Of "Cherry Pie Chompin' Champ"

by Cynthia Hagley

"It all started with an excess of doughnuts at a Biology Club dance." That is how Dr. Michelini explained his proficiency in all sorts of eating competition, after winning the cherry pie-eating contest at last week's "Cherry Tree Chop."

He has brought home three trophies for eating the most watermelon in the Lettermen's Shoe Shine Day contest, and four trophies for the cherry pie gorging events. This makes Dr. Michelini the undisputed champion in these gargantuan endeavors.

Dr. Michelini has admitted that his fondness for watermelon knows no bounds, but he adds that cherry pie is not to his liking. Whether his victories were the cause of this, he did not say. With this secret out, it should be easy for any future competitor to win out over Dr. Michelini.

As for the start of it all, he recalls a Biology Club dance at Thanksgiving time in 1956. The refreshments were cider and doughnuts. Owing to bad weather the crowd at the affair was quite small, leaving a plentiful supply of doughnuts that no one seemed interested in consuming.

When the chaperons were approached with this problem, Dr. Michelini, as one of them, suggested a doughnut eating contest and volunteered to compete. From then on he has served religiously in any such contests. Here may be added that this was the first and last eating bout that he ever lost.

Rumor also has it that the students will be seeing him on top of a four-legged obstinate creature within the next few weeks. All those who saw him in such a posi-



Dr. Francis Michelini

tion last year will remember that he was the leading scorer for the faculty team.

IMMUNE RESPONSES TO
RAGWEED POLLEN
ANTIGENS

SHELDON G. COHEN, M.D.

and

FRANCIS J. MICHELINI, Ph.D.

Wilkes-Barre, Pa.

With the Technical Assistance of

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Reprinted from

THE JOURNAL OF ALLERGY
St. Louis

Vol. 29, No. 5, Pages 446-458, September, 1958

(Printed in the U. S. A.)

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IMMUNE RESPONSES TO RAGWEED POLLEN ANTIGENS

SHELDON G. COHEN, M.D.,* AND FRANCIS J. MICHELINI, Ph.D.,**
WILKES-BARRE, PA.

WITH THE TECHNICAL ASSISTANCE OF LESLIE P. WIENER AND JEROME STEIN***

INTRODUCTION

STUDIES with ragweed pollen, utilizing such analytic techniques as chemical fractionation,¹⁻³ ultracentrifuge,⁴ electrophoresis,⁵⁻⁹ chromatography,¹⁰⁻¹² ion exchange resins,¹⁴ and serologic responses to experimental immunization,¹⁵⁻¹⁶ have indicated the presence of multiple and distinct fractions within the pollen grain. Comprehensive reviews of this subject have been presented recently by Wodehouse¹⁶ and Augustin.¹⁷ The biologic activity of some of these pollen fractions, determined by their ability to elicit positive cutaneous test reactions of reaginic sensitivity in human beings, has been demonstrated. However, their exact roles as antigens and immunologic participants in the production of clinical manifestations of ragweed pollen allergy are still poorly understood.

Among other unanswered questions related to the problem of pollen sensitivity is the highly variable clinical response of patients treated with pollen extracts in attempts at hyposensitization. The value of ascertaining and understanding possible relationships between these two factors—that is, the biologic activity and the antigenicity of individual pollen constituents present in extracts available for treatment purposes—is self-evident. It is possible that in some patients one of the reasons for a lack of effective therapeutic response to specific pollen hyposensitization injections may be associated with a failure to incorporate the prerequisite qualitative, as well as quantitative, intracellular antigenic constituents of the intact pollen grain in treatment solutions. There has been no clear-cut demonstration that the current standard techniques of extraction result in soluble preparations which duplicate the antigenic pattern of their particulate pollen precursors.

An attempt to study this question has been one purpose of this report. This problem has been initially approached by attempting to study the immune response of experimental animals to ragweed pollen and derivative preparations. The antisera were employed in precipitin tests utilizing agar gel diffusion techniques to resolve each precipitin reaction into component antigen-antibody

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This investigation was supported (in part) by a research grant (No. E-1343) from the National Institute of Allergy and Infectious Diseases, Public Health Service.

Received for publication Jan. 24, 1958.

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systems and to serve as an indicator of antigenic constituents of the ragweed preparations. This experimental approach is similar to that which we had previously employed in the study of bacterial intracellular antigens.¹⁸

The second purpose of this study has been concerned with an attempt to determine the ability of ragweed pollen to participate in those in vivo antigen-antibody reactions capable of inducing tissue manifestations of vascular sensitization. In this approach, the technique employed has been that of passive sensitization followed by subsequent challenge with specific antigen. This experimental procedure has been previously described in a demonstration that lesions of pulmonary periarteritis and panarteritis in experimental animals can arise from induced hypersensitivity reactions to foreign sera¹⁹ and to bacteria or their metabolic products.²⁰⁻²¹

EXPERIMENTAL METHODS AND RESULTS

Ragweed Antigens and the Production of Immune Sera.—Series of adult male rabbits were each immunized with one of the ragweed preparations described below, which was derived from the pollen of ragweed (*Ambrosia elatior*).

Each animal received a total dosage of 15 mg. protein nitrogen.* All injections by intravenous, intraperitoneal, or subcutaneous routes were given in fifteen equally divided doses over a twenty-one-day period, and the antiserum was prepared from blood drawn by cardiac puncture seven days after the last injection. In the case of intramuscular injections, the total dosage of ragweed antigen was adsorbed on aluminum hydroxide cream adjuvant²² and administered in multiple sites in the gluteal areas on the same day, and antiserum was prepared from blood drawn by cardiac puncture twenty-one days later.

Immunizing ragweed antigens:

1. *Pollen hydrochloride* (ball-mill-ground whole pollen that had been first converted to pollen hydrochloride²³ according to the method of Roekwell²³). This preparation was administered either by the intramuscular route or by a series of alternating intravenous and subcutaneous injections.

2. *Pollen cytoplasm* (solutions and suspensions of fragmented pollen grains that had been subjected to the disintegrating effects of either sonic waves at 10,000 cycles per second or ultrasonic waves at 800,000 cycles per second†). This resulted in a disruption of the celline and cell walls and subsequent release of intracellular pollen cytoplasm. This preparation was administered by two different techniques: (a) adsorbed on aluminum hydroxide cream and given by intramuscular injections and (b) a series of alternating intravenous and subcutaneous injections.

3. *Pollen extracts* (conventional saline extracts of ragweed pollen prepared by accepted methods of extraction, utilizing alkaline saline extracting

*Nitrogen determinations on all extracts and materials employed in this study were done at Center Laboratories, Port Washington, New York, through the courtesy of J. George Center.

†Delapan, supplied by Hollister-Stier Laboratories, Spokane, Washington, through the courtesy of Dr. E. L. Foubert, Jr.

‡Ultrasonic wave treatment of pollen was conducted through the courtesy of Dr. John M. Ward, Department of Biology, Temple University, Philadelphia, Pennsylvania, and under his direction.

fluid [Coca]). Extracts employing the following variations in preparation were utilized: (a) preliminary defatting of pollen with ether and (b) raw, nondefatted pollen; (c) addition of glycerin to extracting fluid; and (d) plain nonglycerinated. These extracts were administered to different series of animals by (a) intramuscular injection after adsorption of the extract on aluminum hydroxide cream or (b) a series of alternating intravenous and subcutaneous injections.

4. *Whole pollen grains* (a suspension of whole pollen grains, in an amount calculated to contain 15 mg. protein nitrogen, suspended in aluminum hydroxide cream and administered by intramuscular injections).

5. *Whole pollen subjected to multiple extraction.* Whole pollen grains, after thorough defatting treatment with ether, were subjected to repeated suspensions in alkaline saline extracting fluid (Coca) two to four times weekly. Following each extraction the supernatant fluid was tested for protein content and discarded, and the residual pollen was resuspended in a fresh supply of extracting fluid. This procedure was repeated over a period of two months until the supernatant fluid gave only a faintly positive test and the pollen mass had been reduced to one-fifth its original weight. This residual pollen, in an amount equivalent in weight to that used in whole pollen grains, was suspended in aluminum hydroxide cream and given by intramuscular injections.

6. *Pollen oil.* After treatment of the pollen with ether, the ether-soluble portion was filtered off, the ether was allowed to evaporate, and the residual pollen oil was added to liquid Albolene. Each rabbit received a total volume of 15 ml. of the oil-Albolene mixture prepared to contain that amount of pollen oil recovered from 15 grams of whole pollen grains. This was administered in fifteen equally divided doses by a series of alternating intraperitoneal and subcutaneous injections. Serum was prepared from blood drawn by cardiac puncture fourteen days after the last injection. The development of cutaneous sensitivity to pollen oil was determined by the appearance of an area of cutaneous erythema twenty-four to forty-eight hours following the application of the pollen oil in acetone to a shaved area of skin on the back or flank.

TABLE I. NUMBER OF BANDS OF PRECIPITATE FORMED IN THE INTERACTION OF RABBIT ANTISERUM TO RAGWEED POLLEN GRAINS AND TO DERIVATIVE PREPARATIONS WITH SOLUBLE RAGWEED POLLEN EXTRACTS AND CYTOPLASM IN AGAR GEL DIFFUSION PLATES (OUCHTERLONY TECHNIQUE)

RABBIT RAGWEED ANTISERUM TO:	RAGWEED ANTIGENS		
	EXTRACT OF RAW POLLEN GRAINS	EXTRACT OF ETHER-TREATED POLLEN GRAINS	SOLUBLE GRAIN CYTOPLASM (ULTRASONIC PREPARATION)
Ground pollen hydrochloride (Delapan)	1-2	1-2	1-2
Whole pollen grains	6	4-6	3-4
Saline extract and glycerine	4	3-4	2-3
Saline extract	2-3	2-3	1-3
Soluble cytoplasm (ultrasonic preparation)	1-2	2	1-2
Whole pollen grain defatted and hypoproteinized	3	2-3	2-3
Ether-soluble pollen oil	1	1	1

Precipitin Tests.—Tests for the detection of precipitin reactions between ragweed antigens and antisera to ragweed were carried out in agar-filled Petri dishes according to the diffusion technique described by Ouchterlony.²⁴ The soluble ragweed preparations and the antisera were placed in oppositely located wells within the agar to allow for their reaction while diffusing through the gel. Since each band of precipitate represented an independent antigen-antibody system, these precipitin reactions permitted the resolution of reactants into individual components. Double diffusion tests were employed to determine reactions of identity or nonidentity between the ragweed preparations for an evaluation of immunologic similarity or differences between their antigenic constituents.

The results of these precipitin tests are summarized in Table I.

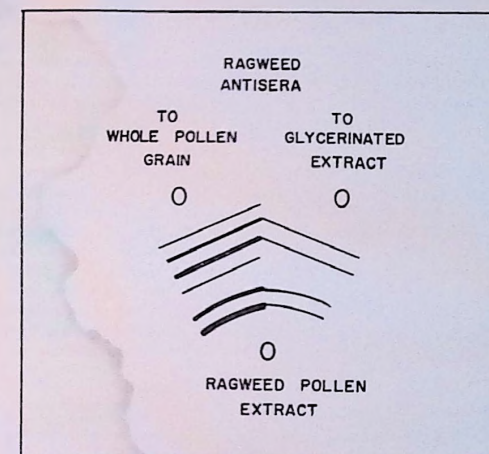


FIG. 1.—Diagrammatic reproduction of precipitin reactions occurring in an agar gel plate by the double diffusion technique of Ouchterlony employing tests with a saline ragweed pollen extract and two rabbit antisera: (1) to whole ragweed pollen grains and (2) to a glycerinated ragweed pollen extract.

In reactions of identity where bands of precipitate were common to antisera to both the whole pollen grain and pollen extracts or soluble pollen cytoplasm in equal amounts, the precipitin bands resulting from participation of the antiserum to the whole pollen grain were much heavier (often by five to ten times) than the corresponding common band (Fig. 1).

The antibody component of antiserum to the pollen oil was shared in common with antiserum to both the whole pollen grain and the pollen extract (Fig. 2).

Passive Sensitization.—Detailed discussions relative to procedure, interpretation, and description of the type of vascular lesions produced by the technique of passive sensitization and challenge employing foreign sera¹⁹ and bacterial antigens²⁰⁻²¹ are reported elsewhere. Briefly, this consists of the administration of high-titer homologous species antiserum into the marginal ear vein of a rabbit. After a thirty-minute incubation period allowing for circulation, distribution, and fixation of the antibodies, the animal is challenged with the intravenous administration of a previously determined subanaphylactic dose of

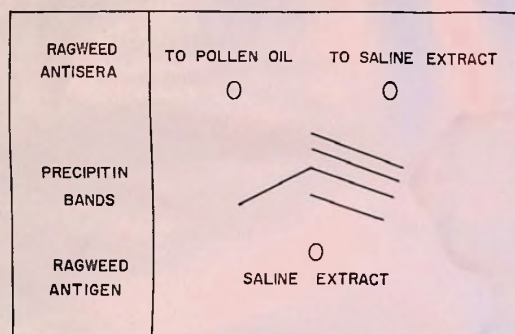


Fig. 2.—Diagrammatic reproduction of precipitin reactions occurring in an agar gel plate by the double diffusion technique of Ouchterlony employing tests with a saline pollen extract and two rabbit antisera: (1) to the ether-soluble fraction of ragweed pollen grains ("pollen oil") and (2) to a saline ragweed pollen extract.

specific antigen. This is divided into aliquots of 1/15, 2/15, 3/15, 4/15, and 5/15 of the total dosage, and each dose is administered at fifteen-minute intervals. Twenty-four hours later the animals are sacrificed by air embolism, and sections of internal organs are taken for study. The most constant lesion uniformly found is that of a pulmonary arteritis (described below). In the present study we attempted to extend this technique to include passive immunization with the rabbit antisera to ragweed pollen and subsequent challenge with ragweed pollen antigens whose methods of production and properties have been described previously in this report. All animals were male albino rabbits of the New Zealand strain, ranging in weight from 1.5 to 2.5 Kg.

Series 1 (Experimental Group): Six rabbits received 5 ml. each of a high-titer antiserum to ragweed pollen and were subsequently challenged with the intravenous administration of a total dosage of 0.15 mg. protein nitrogen ragweed pollen saline extract according to the procedure described above. In five of these six rabbits, hematoxylin-and-eosin-stained sections of the lungs studied microscopically revealed the typical lesions of periarteritis and panarteritis (Fig. 3). The small pulmonary arteries and the pulmonary arterioles showed marked

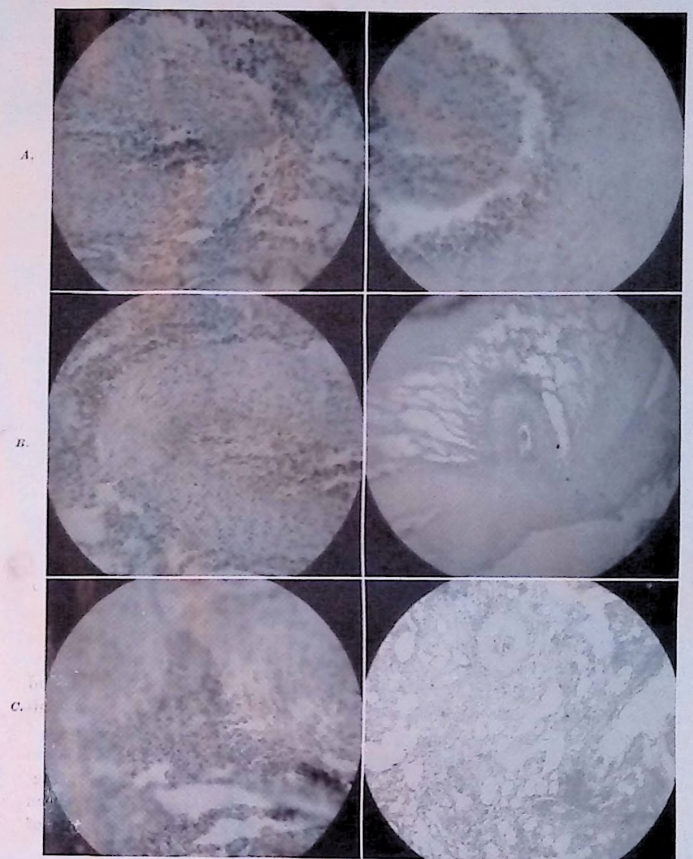


Fig. 3.—Sections from lungs of rabbits that had been passively sensitized with homologous species antiserum to ragweed pollen and subsequently challenged with soluble ragweed extracts twenty-four hours previously. These show involvement of pulmonary arteries and arterioles in varying degrees of arteritis. A and B show high-power magnifications of adventitial edema and cellular infiltration with eosinophils predominating and, to a very much lesser extent, with polymorphonuclear leukocytes and an occasional mononuclear cell. In A, B, and D there is involvement of the intima with edema, some early intimal cell proliferation, and infiltration with eosinophilic granular cells. Collections of eosinophils are seen within the lumen of the vessels shown in A and B, and in D there is an early thrombus formation containing eosinophils. In the lower left field of the vessel shown in A there is disruption in the integrity of the internal elastic membrane. A nodular collection of eosinophils is seen within the adventitia and penetrating into the media of the vessel seen in C. Low-power magnification shows a mild perivascular reaction of the branching vessel shown in E. At the upper pole of the branching vessel shown in F there is a diffuse nodular collection of eosinophils in the adventitia and extending into the adjacent alveolar tissue.

perivascular ringings and adventitial infiltration, especially of eosinophils, with few polymorphonuclear leukocytes and only an occasional lymphocyte and mononuclear cell. Eosinophils were also found infiltrating the media and intima of the arteries, and in several instances collections of eosinophils were found within the lumina of these vessels. Varying degrees of hyperplasia of the endothelial cells of the intima and adventitial edema were also found.

Series 2 (Control Group A): Six rabbits each received only 5 ml. of anti-serum to ragweed pollen by the intravenous route, and in none of these animals could microscopic pathologic lesions of the vasculature be demonstrated.

Series 3 (Control Group B): Each of six rabbits received only ragweed pollen saline extract intravenously in divided dosages totaling 0.15 mg. protein nitrogen. Microscopic pathologic vascular lesions were absent in all six of these animals.

DISCUSSION

The nature of some immunologic factors associated with the precipitation of rabbit antisera to ragweed in specific in vitro antigen-antibody reactions has been studied by Bukantz and associates,²⁵ who applied quantitative precipitin techniques, and by Portnoy and Sherman,²⁶ who employed complement fixation procedures. Thus, it has been possible to utilize immune sera in evaluating such poorly understood points as the effect of storage and preservatives on ragweed extracts²⁵ and the inhibiting effects of the blocking antibody.^{26, 27} Becker and Munoz¹⁵ and Wodehouse¹⁰ have employed rabbit antisera in agar gel diffusion precipitin techniques in attempting antigenic analysis of ragweed pollen extracts, and Wodehouse²⁸ further suggests their application in standardization of pollen extracts. Although these reports have established the validity of interpreting ragweed antigen-antibody precipitin reaction bands in an agar gel, the results of our studies tend to demonstrate the many variables inherent in the qualitative antibody components of rabbit anti-ragweed sera. These apparently are partially dependent upon the particular type and state of the ragweed preparation employed in immunization procedures. Differences were noted in the uniformity of test patterns resulting from the participation of antisera that had been produced against standard pollen extracts and the participation of antisera to the whole pollen grain. This points to the need for a clearer understanding and interpretation of the qualitative antigenic analysis of native ragweed pollen as a prerequisite for the use of animal precipitating antisera in pollen extract standardization.

The precipitinogenic activity of immune rabbit serum can lend itself well to a biologic function as an indicator of antigenic components of the ragweed pollen grain and derivative preparations. However, there is no attempt to compare the rabbit's immune response to ragweed pollen with those still poorly understood conditions associated with the state of natural reaginic hypersensitivity in man or with his reaction to induced measures of hyposensitization that result in the production of blocking serum antibodies. Wodehouse¹⁶ feels that

some of the antigens represented by precipitation bands have been identified with a fair degree of certainty as those biologically active antigens revealed by chemical fractionation, electrophoresis, and chromatography. Augustin¹⁷ has noted that the experimental evidence furnished by clinical trial, skin reactivity of dissolved washed floccules, and correlation of skin tests with diffusion tests of extracts points to, but does not prove, the premise that the grass pollen allergens of man are the precipitin-producing antigens of the rabbit.

The exact number of biologically active fractions within the ragweed pollen grain has not been uniformly determined, and results of fractionation procedures have varied with methods employed. The total number of fractions reported has varied from two or three, as first determined by chemical fractionation,¹ upward to eleven or twelve with the employment of newer techniques utilizing ion exchange resins¹⁴ and chromatography.¹⁰⁻¹¹ There is insufficient evidence, however, to indicate that all these fractions are antigenic or biologically active. The finding, in the whole pollen grain, of six components, sufficiently antigenic to give rise to individual rabbit serum precipitins roughly parallel the findings of Rockwell,² who isolated five fractions by chemical fractionation; Abrahamson and associates,^{3, 6} who reported the presence of one major and six or more minor fractions in studies employing electrophoresis and the ultracentrifuge; Dankner and his co-workers,¹² who employed paper chromatography and reported six and seven active components; and also those of Becker and Munoz,¹⁵ who noted five antigenic fractions capable of precipitinogenic action with immune rabbit sera in a gel diffusion system with the Oudin technique. Although we employed the same method of precipitin testing as Wodehouse,¹⁶ we were unable in any instance to duplicate his finding of at least eight component antigens in short ragweed pollen extract. Cebra's²⁹ explanation would assume that, because of overlapping of bands and unfavorable concentration of reactants, a submaximal number of bands will be revealed by complex testing mixtures in tests with ragweed extracts containing as many as ten antigens.

The discrepancy in the results of these studies may, of course, be related to the actual technical methods employed. However, other explanations considered include those reports that (1) electrophoretic analysis demonstrated that these fractions may be mixtures⁶ and that some contain common and independent allergens,³⁰ (2) proteins in ragweed extracts gradually break down into fragments of lesser magnitude,⁷ and (3) these fractions show extreme lability which results in fragmentation.¹²⁻¹³ In this regard, our present findings of distinct variations in qualitative antibody content of immune rabbit sera directly related to the type of immunizing ragweed antigen employed would tend to suggest that the mode of preparation of a ragweed extract may affect its qualitative antigenic content.

It is possible that the intact pollen grain may contain more than the maximum number of six detected constituent antigens, intracellularly or within the cell wall. This has been impossible to determine, however, because technical limitations imposed by the technique of gel diffusion preclude the use of any

but soluble antigens. In converting the whole pollen grain to a soluble extract there apparently occurred in at least two of the components a loss in antigenicity sufficient to effect their immunizing potential. The relatively greater antibody content in antiserum to the whole pollen grain over that of antiserum to soluble extracts suggests the antigenic superiority characteristic of most cellular or particulate antigens.²¹ This is similar to the finding of greater antibody content in antiserum to bacterial cells in comparison with antiserum resulting from immunization with soluble bacterial antigens.¹⁸

It was felt that perhaps the advantages of the enhanced immune response to the native intact pollen grain might be simulated with a more feasible immunization technique utilizing soluble pollen protoplasm. An attempt was then made to recover such cytoplasm by disrupting the integrity of the ectine and cell walls of the intact grain by subjecting pollen to the disintegrating effects of ultrasonic and supersonic vibrations. However, modifications in the integrity of the whole pollen grain by physical factors, whether ultrasonic wave disruption or mechanical grinding, apparently resulted in antigenic alterations characterized by a loss of immune response to as many as four or five active pollen constituents. These effects appear to be similar to those noted following ball mill grinding and/or ultrasonic wave exposure to bacterial cells where antigenic modifications of soluble bacterial cytoplasm have also been demonstrated.¹⁸ This appears to be generally true of most cellular antigens. Following traumatic procedures, different antigens may possibly arise as a result of further splitting and/or structural rearrangement or formation of new combinations of the individual constituents that originally composed the complex but labile molecular aggregates of the intact cell.²²

Generally there was very little difference noted in the respective numbers of antigenic constituents of saline-extracted raw ragweed pollen and that prepared from pollen subjected to preliminary treatment with a fat solvent. Such defatting procedures may therefore result in only minor changes. In an occasional animal, however, a loss of immune response to one or two antigenic components did occur.

The addition of glycerin apparently enhanced the antigenic effect of saline extracts by increasing the qualitative immune response to one or two antigenic components of the pollen grain. It is not known whether this may be due to some adjuvant effect of glycerin or whether, as shown by Bukantz and co-workers,²³ nonglycerinated pollen extracts may deteriorate rapidly in biologic action under storage conditions.

It is interesting to note the extreme difficulty experienced in completely removing antigenic material from ragweed pollen, even after periods of as long as two months of multiple and repeated extractions with a resultant loss of three-fifths to four-fifths of the original weight of the pollen mass. Thus, much valuable ragweed antigen, both qualitative and quantitative, may remain within the whole pollen grain after standard extracting procedures. Rockwell²⁴ had previously estimated that only one-sixth of the active antigen is extracted in the preparation of crude pollen extract.

The presence of circulating precipitating antibody to one major antigenic component of ragweed pollen in the sera of animals injected with the ether-soluble fraction of the pollen grain ("pollen oil") was a surprising finding, in view of the accepted concept of the selective action of proteins and carbohydrates as antigens in such immunologic phenomena. The presence of this serum antibody was not related to an associated development of cutaneous contact sensitivity to the ragweed oil. The fact that this particular antigenic constituent of the pollen oil is shared in common with soluble pollen cytoplasm and pollen extract suggests two possible explanations: (1) that this is really a water-soluble protein constituent taken down in colloidal suspension in an ether-water mixture because of inability to extract the pollen grains under completely anhydrous conditions and (2) that this constituent may be a lipoprotein soluble in ether saline or ether. However, we have been unable thus far to identify it as such by selective precipitin band staining with Sudan black B or oil red R.²² The presence of this common antigen in both ragweed extract and pollen oil may help to explain the unusual finding of both the positive intracutaneous whealing reaction to pollen extract and the positive contact reaction to pollen oil by patch test technique in the same ragweed-sensitive patients.²²⁻²⁴

Thus, it is seen that current accepted methods of extraction in the preparation of ragweed antigens for clinical use in hyposensitization techniques may fall short of duplicating the antigenic pattern of the whole ragweed pollen grain. However, whether this is actually necessary for satisfactory treatment of the ragweed-sensitive patient, together with other undetermined points concerned with an exact identification of those precipitin bands to indicate pollen constituents whose presence is necessary to evoke such an immune response, is not known. If the immune response of an experimental animal is any indication of the antigenic efficacy of a ragweed extract required for human treatment purposes, then the search for feasible techniques for duplicating immunization with all native intracellular pollen antigenic fractions without antigenic alteration or denaturation should be the object of further investigation.

The microscopic finding of lesions of periarteritis and panarteritis of the pulmonary tissue in sensitized and challenged rabbits indicates the vascular sensitizing properties of ragweed pollen in this species. The experimental conditions associated with the production of these lesions suggests that the development of hypersensitivity vasculitis is dependent upon a shock tissue response to the specific interaction of antigen and antibody rather than a toxic action of the ragweed pollen antigens themselves or an *in vivo* effect of their antibodies *per se*. Histologic appearance of these vascular lesions and the immunologic prerequisites for their production simulated in detail the features associated with the experimental production of arterial sensitization observed with other antigen-antibody systems related to foreign sera,²⁵ bacteria, and bacterial products.²⁶⁻²⁷ Thus, there is offered further evidence of the validity of our previous suggestion that the development of pulmonary arteritis in the rabbit may be a tissue manifestation of the subacute anaphylactic reaction and that it suggests the potential of ragweed pollen antigen for participation in hypersensitivity reactions affecting the vasculature.

SUMMARY

1. Series of rabbits were experimentally immunized with either intact grains of whole ragweed pollen or one of its derivative preparations, including saline extracts, soluble cytoplasm obtained by subjecting pollen grains to the disintegrating effects of ultrasonic and supersonic vibrations, ball-mill-ground whole pollen hydrochloride, and ether-soluble fraction ("pollen oil").

2. The antisera obtained from these animals were employed as indicators of antigenic components of ragweed pollen in determinations of patterns of precipitin test reactions employing agar gel diffusion plate techniques (Ouchterlony).

3. The interpretation of antigen-antibody precipitin band patterns suggests the following:

- The maximum number of antigenic components in ragweed pollen that could be determined by this technique was six.
- There is both qualitative and quantitative antigenic superiority in the whole ragweed pollen grain, which could not be duplicated with immunization procedures employing preparations of pollen extracts or soluble pollen cytoplasm.
- Conversion of the whole pollen grain to a soluble extract may result in an antigenic alteration involving as many as two pollen constituents.
- Soluble pollen cytoplasm obtained by disrupting the integrity of the ectine and cell walls of the pollen grain by mechanical procedures may undergo a loss of two or three antigenic components.
- Preliminary defatting of the pollen with ether results in only minor antigenic effects, but the addition of glycerin to a saline ragweed extract enhances its antigenicity.
- Ragweed pollen subjected to standard and prolonged extraction procedures contains effective residual antigenicity.
- Ragweed pollen oil contained one antigenic fraction of undetermined nature capable of inducing a serum precipitating antibody response.

4. In rabbits passively sensitized with homologous species antiserum to ragweed and subsequently challenged with specific soluble pollen extract, microscopic lesions of periarteritis and panarteritis of the pulmonary vasculature were demonstrated. These lesions could not be found in rabbits receiving either the antiserum or the pollen antigen alone. The development of hypersensitivity vasculitis as a tissue response to the in vivo ragweed pollen antigen-antibody reaction is suggested.

REFERENCES

- Stull, A., Sherman, W. B., and Hampton, S. F.: Antigen Fractions in Ragweed Pollen. I. Water-Soluble Fractions, *J. ALLERGY* 12: 117, 1941.
- Sherman, W. B., and Stull, A.: Antigen Fractions in Ragweed Pollen. III. Further Evidence of Immunologic Specificity of Fractions 1 and 2, *J. ALLERGY* 18: 287, 1947.
- Rockwell, G. E.: Active Chemical Components of Crude Ragweed Extracts, *Ohio M. J.* 39: 128, 1943.

- Sanigar, E. B.: Ultracentrifugal Studies of Ragweed Extracts, *J. Franklin Inst.* 230: 781, 1940.
- Abrahamson, H. A., Moore, D. H., and Gettner, H. H.: A General Electrophoretic Pattern in Extracts of Pollens Causing Hay Fever, *J. Phys. Chem.* 46: 1129, 1942.
- Newell, J. M.: Electrophoretic Studies on Chemical Fractionation of Ragweed Pollen Extracts, *J. ALLERGY* 14: 444, 1943.
- Stevens, F. A.: The Quantitative Changes in Various Fractions of the Precipitable Nitrogen in Ragweed Extracts at 37° C., *J. ALLERGY* 18: 90, 1947.
- Abrahamson, H. A.: Chemical, Physical and Immunological Properties of Electrophoretically Purified Pollen Extracts, *Ann. Allergy* 5: 19, 1947.
- Stevens, F. A., Moore, D., and Gelston, T.: The Major Electrophoretic Components of Giant Ragweed Extracts: Molecular Weight, Chemical and Biological Characteristics, *J. ALLERGY* 22: 356, 1951.
- Perlman, E.: Chromatographic Analysis of Ragweed Pollen Extract, *Bull. New York Acad. Med.* 27: 586, 1951.
- Bernstein, T. B., Mosher, A. L., and Mariella, R. P.: A Purification of the Active Principle of Short Ragweed Pollen, *Science* 113: 377, 1951.
- Dankner, A., Bukantz, S. C., Johnson, M. C., and Alexander, H. L.: Fractionation of Pollen Extracts by Chromatography. I. Preliminary Studies With Ragweed Extracts, *J. ALLERGY* 22: 437, 1951.
- Frankel, S., Johnson, M. C., Schiele, A. W., Bukantz, S. C., and Alexander, H. L.: Fractionation of Pollen Extracts. II. Chemical and Paper Chromatographic Investigations of Ragweed Pollen Extracts, *J. ALLERGY* 26: 33, 1955.
- Bookman, R., and Wax, H.: Fractionation of Ragweed Antigens by the Use of Ion Exchange Resins, *J. ALLERGY* 25: 12, 1954.
- Becker, E. L., and Munoz, J.: Multiplicity of Antigens in Ragweed Pollen Extracts as Demonstrated by the Technique of Oudin, *Proc. Soc. Exper. Biol. & Med.* 72: 287, 1949.
- Wodehouse, R. P.: Identification of Ragweed Antigens in Gel Diffusion Precipitates, *Ann. Allergy* 13: 39, 1955.
- Augustin, R.: Chemical, Biochemical and Immunologic Advances in Allergy, With Special Reference to Pollen and Their Standardization, *Quart. Rev. Allergy* 9: 504, 1955.
- Cohen, S. G., Dzury, D. S., and Michelini, F. J.: Immune Responses to *Staphylococcus Aureus* Antigens, *J. Infect. Dis.* (in press).
- Cohen, S. G., Mayer, L. D., and Cripe, L. H.: The Experimental Production of Arteritis by Passive Sensitization, *J. Immunol.* 66: 487, 1951.
- Cohen, S. G., Mokyehe, W. E., and Drapiewski, J. F.: The Etiological Role of *Streptococcus Toxin-Antitoxin* Systems in the Experimental Production of Arterial Sensitization, *J. ALLERGY* 24: 411, 1953.
- Cohen, S. G., Dzury, D. S., and Michelini, F. J.: *Staphylococcus Aureus* Antigens in Hypersensitivity Reactions and Experimental Arterial Sensitization, *J. ALLERGY* 28: 531, 1957.
- Hektoen, L., and Welker, W. H.: Precipitin Production in Rabbits Following Intramuscular Injection of Antigen Absorbed by Aluminum Hydroxide, *J. Infect. Dis.* 53: 309, 1933.
- Rockwell, G. E.: A Whole Pollen Antigen Hydrochloride, *Ann. Allergy* 11: 175, 1953.
- Ouchterlony, O.: Antigen-Antibody Reactions in Gels. IV. Types of Reactions in Co-ordinated Systems of Diffusion, *Acta. path. et microbiol. scandinav* 32: 231, 1953.
- Bukantz, S. C., Johnson, M. C., and Hampton, S.: Quantitative Immunological Studies With Allergens: Colorimetric Absolute Methods in the Analysis of Factors Influencing Precipitation of Ragweed Pollen Extracts and Homologous Rabbit Antisera, *J. ALLERGY* 20: 1, 1949.
- Portnoy, J., and Sherman, W. B.: Complement Fixation Studies in Ragweed Allergy. I. Quantitative Complement Fixation Studies in Ragweed Pollen Extract and Homologous Rabbit Antisera, *J. ALLERGY* 25: 215, 1954.
- Portnoy, J., and Sherman, W. B.: Complement Fixation Studies in Ragweed Allergy. II. Determination of Antibody in Human Sera to Ragweed Antigen by Means of a Complement Fixation Inhibition Test; the Relationship of Antibody so Determined to the Passive Transfer for Blocking Antibody, *J. ALLERGY* 25: 229, 1954.
- Wodehouse, R. P.: Standardization and Antigenic Analysis of Pollen Extracts by Gel Diffusion, *Ann. Allergy* 11: 720, 1953.
- Cebra, J. J.: Starch Block Electrophoresis of Ragweed Pollen Antigens, *J. Immunol.* 79: 118, 1957.

30. Richter, M., Harter, J. G., Schon, A. H., and Rose, B.: Studies on Ragweed Pollen. III. Estimation of the Minimum Number of Allergens in the Water Soluble Extract of Ragweed Pollen and a Critical Evaluation of the Neutralization Technique; the Demonstration of Two Different Reagents in Sera of Ragweed Sensitive Individuals, *J. Immunol.* 79: 13, 1957.
31. Boyd, W. C.: *Fundamentals of Immunology*, ed. 3, New York, 1956, Interscience Publishers, Inc., p. 211.
32. Krongold, L., and Lipari, R.: Immunochemical Studies of Human Plasma Beta Lipoprotein, *Science* 121: 170, 1955.
33. Fromer, J. L., and Burrage, W. S.: Ragweed Oil Dermatitis. *J. ALLERGY* 24: 425, 1953.
34. Cohen, S. G.: Clinical Observations. (To be published.)



IMMUNE RESPONSES TO
RAGWEED POLLEN
ANTIGENS

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and
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Reprinted from
THE JOURNAL OF ALLERGY
St. Louis

Vol. 29, No. 5, Pages 446-458, September, 1958

(Printed in the U. S. A.)

*from the files
of "Dr. Mike"
F. J. Michelini*

February 26, 1960

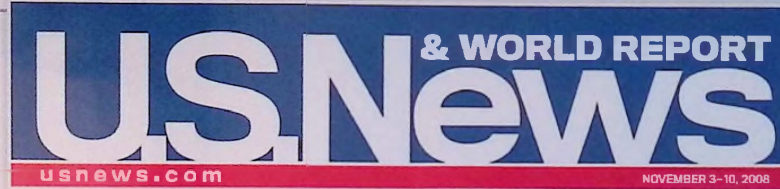
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College Tuition Crisis • America on the Decline?



Breaking Cancer's Code

THE REVOLUTION IN DIAGNOSIS AND TREATMENT

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Illustration of a cancer cell

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STAPLES

LETTER FROM VIRGINIA



The two nuclear reactors at the North Anna power plant

A Tough Nuclear Decision

One rural town ponders a third plant—and making a hot lake even hotter

By Kent Garber

MINERAL, VA.—The area around Lake Anna is pastoral, but it has never been a typical resort community. After all, the lake was built to help cool two commercial nuclear reactors. Residents who live along the lake blithely talk about its “hot side” and “cold side,” depending on proximity to the power plant, while nearby, the owners of the award-winning Lake Anna Winery joke affectionately about their wine “glowing after dark.”

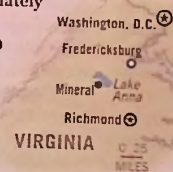
For a community that's lived for the past 30 years with reactors in its midst, getting a third one might not seem like a big deal. That's what Dominion, the utility company that operates the existing two units, is hoping as it lays plans to build another one here. At the moment, Dominion is one of the leading applicants in a growing wave of companies hoping to revive America's long-stalled nuclear industry.

Comeback. If Dominion's proposed Virginia plant clears the remaining state and federal regulatory hurdles, it could become the first nuclear plant approved for construction in three decades in the United States. Dominion has even

picked out the spot—a large plot situated between the two reactors on one side and transmission lines on the other.

The only thing that's gone in the ground so far, however, is a set of sticks: wooden stakes marking an intention. With today's high energy prices—and mounting concern about climate change—there are plenty of reasons why nuclear power seems poised for a comeback. And yet, as Eugene Grechek, Dominion's vice president of nuclear development, said on a recent afternoon as he stood looking across the site, “There's no guarantee that this reactor will get built.” The earliest that official approval could come is 2011, which means the plant couldn't start operating until 2016 at the earliest.

Nuclear veterans like Grechek tend to speak cautiously when they talk about a nuclear revival, and it's not hard to see why. They all remember what happened after the 1979 accident at Three Mile Island near Harrisburg, Pa., when a reactor suffered a partial meltdown. In the years that followed, dozens of nuclear projects were abandoned, bogged down by costly safety requirements, as well as lawsuits that delayed regulatory approval and drove costs even higher. In the meantime, the reactors still operating in this country (104 at last count) have continued to chug along, producing nearly 20 percent of the nation's elec-



tricity. Yet given the dramatic rise in electricity projected over the next 20 years, experts say it will take 35 to 40 new reactors just to keep that figure flat.

Getting one reactor approved and built will be a long enough—even with recent federal efforts to streamline regulatory approval and the support that exists in a pocket of central northern Virginia. If most people see nuclear power as a prominent national issue, it's not clear, an intensely local one. Aside from the area around the lake, Louisa County is relatively poor and has an hour traffic is six or seven cars at a stop sign. Down the road regularly, and agriculture—corn, mainly—is still the main occupation.

Officials here are well aware of the money that existing reactors, built in the late 1970s, have put in the region. Last year, they generated roughly \$11 million in tax revenues, which goes toward schools and other services. More than 900 people are employed at the plant. A third reactor would create about 3,000 temporary jobs during the five years it would take to build it and some 750 permanent jobs to operate it. It should come as no surprise, then, that officials here enthusiastically support the project.

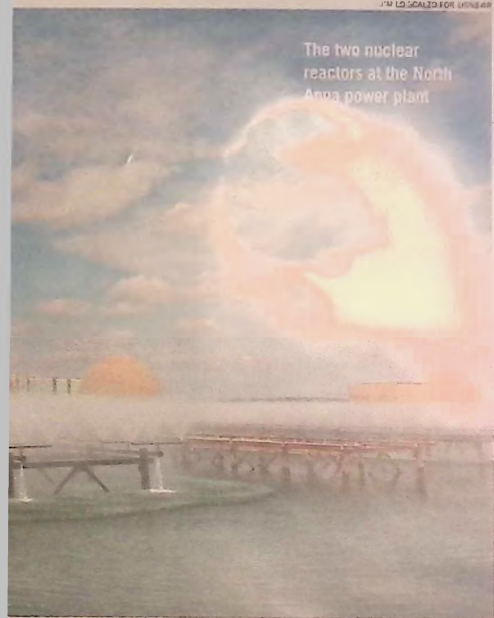
Most Louisa County residents, however, profess not to care much one way or the other if the third reactor gets built. Safety concerns barely rate a mention. As Patty Chandler, a cashier at Dickinson's convenience store a few miles down the road from the power plant, says: “I don't care about two reactors. If one of them goes, we're in bad luck. So what's the big deal about a third one?”

Hot water. Some lake residents, though, are more worried. Lake Anna is unusually small for a nuclear reactor, let alone a single nuclear reactor, let alone a single nuclear reactor, let alone a single nuclear reactor. Water is constantly flowing, pushing the discharge plant downstream. Lakes, however, are more so, in Lake Anna's case, the hot water that the plant ends up staying around for a while.

This has created two problems. On the one hand, the lake receives the plant's discharge, the sun heats the water already, can reach above 100 degrees. Even in the cooler months, in February and March, it stays warm. Boaters bunched up in heavy jackets often see people playing in the water in winter. The second problem is the water level. Between the drought and the reactors' thirst for water, some recent summers have fallen several feet, exposing stumps or rocks that endanger boaters.

A third reactor would strain the lake further. It has become a sore point between Dominion and the 2003 Dominion report suggested that water

The two nuclear reactors at the North Anna power plant



Nuclear Decision

—and making a hot lake even hotter

picked out the spot—a large plot situated between the two reactors on one side and transmission lines on the other.

The only thing that's gone in the ground so far, however, is a set of sticks: wooden stakes marking an intention. With today's high energy prices—and mounting concern about climate change—there are plenty of reasons why nuclear power seems poised for a comeback. And yet, as Eugene Grechek, Dominion's vice president of nuclear development, said on a recent afternoon as he stood looking across the site, "There's no guarantee that this reactor will get built." The earliest that official approval could come is 2011, which means the plant couldn't start operating until 2016 at the earliest.

Nuclear veterans like Grechek tend to speak cautiously when they talk about a nuclear revival, and it's not hard to see why. They all remember what happened after the 1979 accident at Three Mile Island near Harrisburg, Pa., when a reactor suffered a partial meltdown. In the years that followed, dozens of nuclear projects were abandoned, bogged down by costly safety requirements, as well as lawsuits that delayed regulatory approval and drove costs even higher. In the meantime, the reactors still operating in this country (104 at last count) have continued to chug along, producing nearly 20 percent of the nation's elec-

tricity. Yet given the dramatic rise in electricity use that's projected over the next 20 years, experts say it will take at least 35 to 40 new reactors just to keep that figure from dropping.

Getting one reactor approved and built will be challenge enough—even with recent federal efforts to streamline regulatory approval and the support that exists in this small pocket of central northern Virginia. If most people see nuclear power as a prominent national issue, it remains, at its core, an intensely local one. Aside from the area right around the lake, Louisa County is relatively poor and rural. Rush-hour traffic is six or seven cars at a stop sign. Tractors putter down the road regularly, and agriculture—corn and hay farming, mainly—is still the main occupation.

Officials here are well aware of the money that the two existing reactors, built in the late 1970s, have pumped into the region. Last year, they generated roughly \$11 million in local tax revenues, which goes toward schools and road maintenance. More than 900 people are employed in high-paying jobs at the plant. A third reactor would create about 3,000 temporary jobs during the five years it would take to build it and some 750 permanent jobs to operate it. It should come as no surprise, then, that officials here enthusiastically support the project.

Most Louisa County residents, however, profess not to care much one way or the other if the third reactor gets built. Safety concerns barely rate a mention. As Patty Chandler, a cashier at Dickinson's convenience store a few miles down the road from the power plant, says: "We already have two reactors. If one of them goes, we're in bad shape anyway. So what's the big deal about a third one?"

Hot water. Some lake residents, though, are understandably more worried. Lake Anna is unusually small as a source of water to cool a single nuclear reactor, let alone three. Most nuclear plants are built along rivers or oceans where the water is constantly flowing, pushing the discharge from the plant downstream. Lakes, however, are more slow moving, so, in Lake Anna's case, the hot water that comes out of the plant ends up staying around for a while.

This has created two problems. On the lake's warm side, which receives the plant's discharge, the summer water temperature already can reach above 100 degrees Fahrenheit. Even in the cooler months, in February and March, the water stays warm. Boaters bundled up in heavy jackets and caps often see people playing in the water in swimsuits. The second problem is the water level. Between the region's ongoing drought and the reactors' thirst for water, lake levels in some recent summers have fallen several feet, in some places exposing stumps or rocks that endanger boats.

A third reactor would strain the lake further, which has become a sore point between Dominion and lake residents. A 2003 Dominion report suggested that water temperatures

near the reactor might rise as high as 113 degrees in the summer with three reactors operating. "That shocked a lot of people," says Doug Smith, a retired government worker who owns a farm in Louisa County and a boathouse on Lake Anna. In response, Dominion spent several months designing an alternative system to release water into the air rather than back into the lake. It's still working to address water-level issues.

Such delays are costly, and cost is one of the main reasons the nuclear industry has struggled—and continues to struggle—in the United States. Industry insiders estimate that it will cost \$6 billion to build the third reactor, assuming everything goes according to plan. Work on the project began in 2000, and Dominion has spent the past five years doing safety and environmental reviews. Construction will take at least five years, and each year of delay could add at least \$1 billion, maybe more, to the final bill. It's that estimated price tag, above all, that will determine whether Dominion's board of directors ultimately decides to give the reactor the go-ahead.



Virginia's Lake Anna remains a popular destination for boaters.

Some of the other major flash points about nuclear energy that tend to dominate the political debate—where to store nuclear waste, how to safeguard power plants from terrorist attacks—have figured less prominently here, although they're not entirely absent. Like most companies, Dominion stores its spent nuclear fuel on-site, in off-white-colored casks in a secured area. The casks don't take up much space, but they do cause consternation, especially among environmentalists. "Dominion

has gotten its way here for more than five decades now," says Lou Zeller, president of the Blue Ridge Environmental Defense League, a regional antinuclear organization.

Zeller's group is currently involved in a separate legal dispute with Dominion over the company's plans for disposing of "low-level waste" like workers' clothing that contains smaller amounts of radiation. Dominion used to send that material to a storage location in South Carolina, but that site, as of this past summer, no longer accepts it. So Dominion will most likely have to store that on-site, too.

Though concerns about nuclear waste were raised last spring at a public hearing at Louisa County High School, many residents who attended say such objections came primarily from outsiders—"people from Washington and Charlottesville with agendas to push," says Bill Murphey, a lake-side resident. They do, however, expect their community to experience other impacts. They say they'll need more schools, roads, and homes to accommodate workers and their families during the lengthy construction process.

Some locals, noting that the schools here are already overcrowded, fear the county hasn't done enough to prepare should the reactor get built on schedule. On the other hand, given that a new reactor hasn't been approved since 1978, it could still be many years, if ever, before construction begins. •

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Unlocking the S

The struggle against cancer has failed too many people. But there's now real hope of changing that

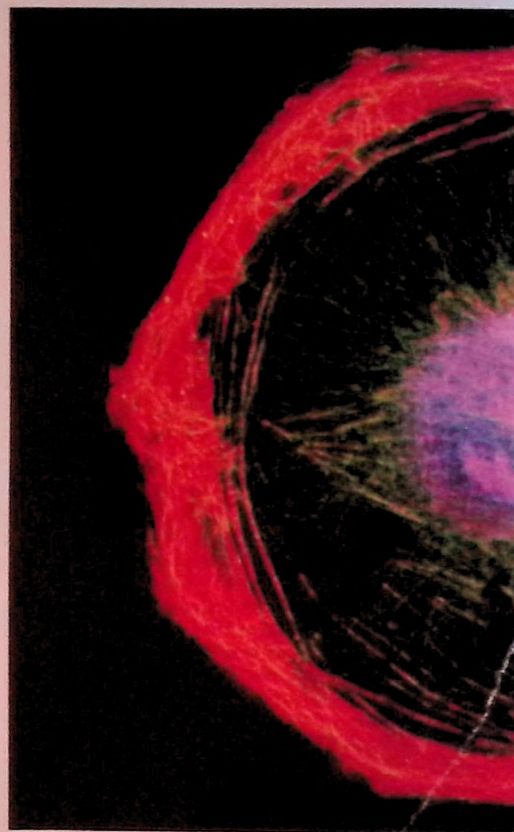
By Bernadine Healy, M.D.

Imagine cancer, 2040. A 45-year-old woman who has never smoked develops lung cancer, which today kills more people than any other kind. (By then, of course, cigarettes will have gone the way of the buggy whip, and lung cancer rates will be cut in half.) She undergoes outpatient surgery, and her doctors quickly scrutinize the tumor's genes and feed the data, along with other information from her electronic medical record, into a desktop computer that crunches out a treatment plan all but certain to work. At subsequent checkups, her blood is tested for the earliest hint of a tumor recurrence—though were such news to come, it would bring no doom and gloom. Her doctor would simply analyze a few of the cells that even the tiniest tumors shed and prescribe a suitable next round of therapy. Throughout, her busy life is barely interrupted, and her hair stays wonderfully intact.

Sound like pure fantasy? It isn't.

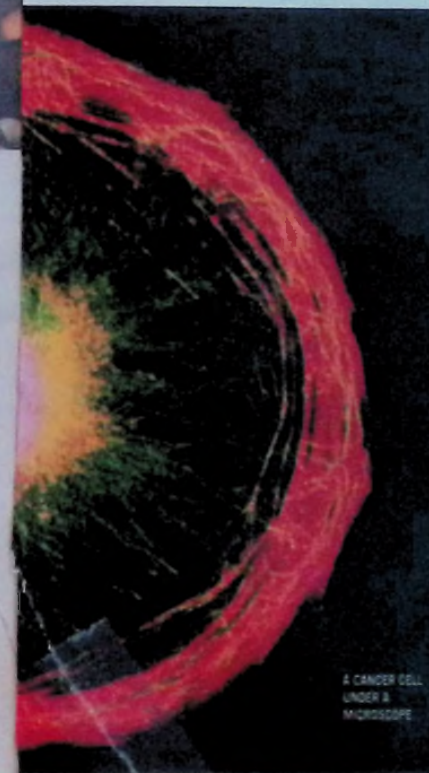
Yes, cancer as we know it remains a fearsome foe, killing well over half a million Americans each year despite the well-funded, 40-year-old war against it. It still erupts in healthy tissue out of the blue, and—programmed to grow perpetually—mercilessly invades surrounding tissue and spreads to distant sites. Surgery and radiation and chemotherapy can stymie its growth and, if still early, cure it altogether. But they can ravage healthy tissue, too, and all too often the tumor is deadly by the time it appears. Advanced disease brings discouraging failures as often as not. Most patients facing invasive cancer get vague guesses as answers to questions like “Will I respond to this treatment?” “Will my tumor come back?”

Epiphany. But thanks to an epiphany in medicine about the very nature of cancer, most oncologists now believe in a not-too-distant future where cancer will be curable or a manageable chronic disease. What has changed radically in recent years is the view that cancers of different types—breast, lung, colon—are relatively homogeneous based on how they look under the microscope and how far they have burrowed into the body. In fact, even malignancies that seem identical are highly complex and varied where it counts—in their genetic makeup. This reality dictates an entirely new approach to diagnosis and treatment: “personalized medicine,” which looks to a patient's unique genetic differences to better understand his or her illness and tailor care.



Stephen Jay Gould, the late Harvard biologist, fathomed the implications of biological variation in 1985, ahead of his time, when he wrote “The Median Isn't the Message,” an essay pointing out that cancer survival statistics tend to obscure a wide range of patient outcomes. Gould, diagnosed with a deadly cancer at age 40, faced a gloomy median survival time of just eight months. But he decided that optimism was justified when he realized that “the median” meant that half the people with his tumor survived longer. Indeed, he lived happily for 20 more years. As a scientist, Gould surely would have loved to have had access to technology that could have given him a better idea of what lay ahead.

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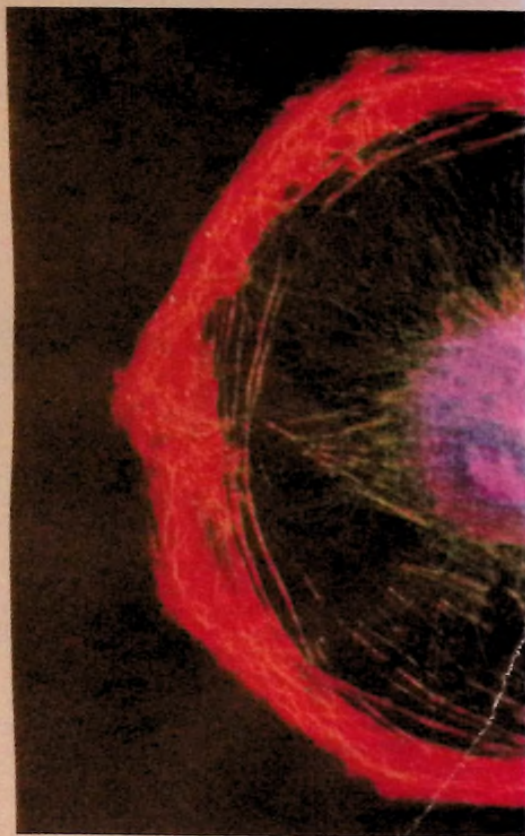


A CANCER CELL UNDER A MICROSCOPE

That powerful technology, which so rapidly and successfully mapped out all 20,000 genes of the human genome, is now being aimed at decoding the genetic underpinning of cancer. The sheer volume and variability of mutations resulting in interconnected biological processes contribute to malignancies are mind-boggling, but the Cancer Genome Atlas project, a program underway at the National Institutes of Health since 2006, is a monumental step toward cancer. Its goal is to map the full genome—the comprehensive instruction book—of virtually all human cancers.

What does this really mean for individual patients might ask? A lot. A reference manual that catalogs the

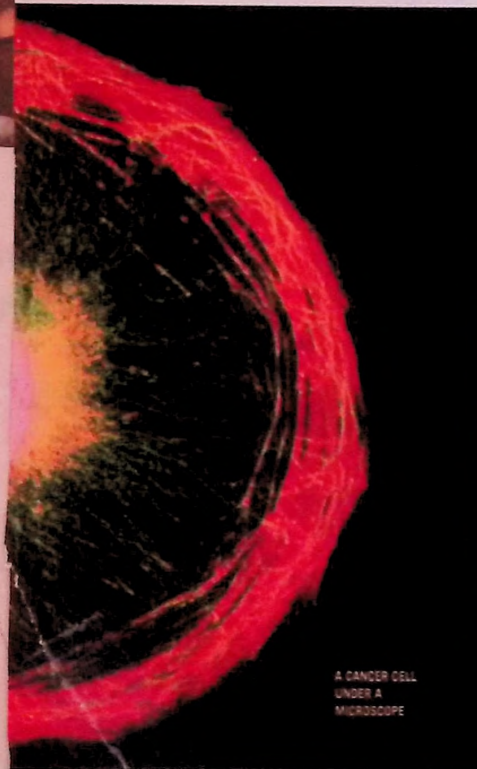
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DAVID SICKLER—GETTY IMAGES

ecrets of Cancer



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versity of the genetic mix underlying individual cancers sets the stage for flagging a tumor's characteristics and behavior—witness our 2040 lung cancer patient—and offering personalized treatments. The mighty Atlas has already shown impressive results. In September, researchers reported the project's first findings, the gene profile of the most common and deadliest form of brain cancer, glioblastoma. Not only did researchers catalog the major known DNA blunders in this disease, but they also provided actionable insights into why gliomas are so often resistant to conventional chemotherapy. And Atlas uncovered three significant and previously unreported mutations, common to most of the more than 200 tumors analyzed, that point to new targets for treatment.

Comprehensive genomic information of this kind will also be critical to evaluating new therapies. And here we have to humbly admit a gaping deficit: The gold standard of today, the randomized controlled clinical trial, assumes that randomly chosen test subjects have identical tumors, based on the old thinking. That just doesn't work for cancer anymore. If in fact those tumors are markedly different from one another, little wonder that—as Gould reminds us—a drug that extends life a mere two months on average fails many people but gives precious years of life to some. Better answers would come from smaller patient studies comparing genetically matched cancers. Such trials would be faster, cheaper, and vastly improved for all patients—both for those who should, and should not, partake in the new therapy.

Next steps. The path to cancer 2040 demands coordinated and cooperative efforts by researchers spurred on by goals and timetables not unlike the ones that made the mapping of the human genome so famously successful. Among the next big challenges: Study and harness the cell's natural instincts to self-destruct when it senses deranged DNA. Learn the wily ways of cancer stem cells, which in small but powerful numbers seem to initiate tumors and just may be the brains behind cancer's perpetual growth. Find the way to consistently manipulate the immune system to attack and destroy even disseminated cancers, as it has done on rare occasions to bring nearly miraculous cures. And finally, enlist engineers and computer scientists to embrace the grand challenge of personalized medicine, as recently proposed by the National Academy of Engineering. What's needed, for example, are tools to readily store, analyze, and use massive amounts of genomic data, and gene chips and sensors to find and track cancers early, anywhere in the body.

As the following pages make clear, the possibilities of discovery are rich and at hand. And the urgency is evident in the faces of patients who yearn for better. It will take flexibility, organization, and heart to get there, but it can happen—perhaps long before 2040. ●

COVER STORY

A Radical Rethinking of Treatment

The aim is to make cancer curable or a manageable chronic disease

By Katherine Hobson

The hoary metaphor of the war on cancer, as overused as it may be, is as evocative as ever to describe our efforts to beat the disease that will claim nearly 566,000 American lives this year alone. So let's fall back on martial imagery to describe our current position: We now know the enemy far better than ever before. And that promises much more precise targets.

Of course, as even Sun Tzu recognized, intel must be translated into effective action, and in this struggle, that effort has been all too plagued by failure. But mounting discoveries about the astoundingly complex essence of cancer—that its causes lie in multitudes of genes gone awry—are also pointing the way toward treatments aimed in laserlike fashion at a patient's unique set of genetic glitches. In mid-October, scientists reported in *Nature* that they've identified 26 key mutated genes linked to lung cancer, for example. "All of the pieces of the puzzle are in front of us," says Martin McMahon, a cancer biologist at the University of California-San Francisco Helen Diller Family Comprehensive Cancer Center. "It used to be that 10 percent of the pieces were in front of us and the other 90 percent were in a box under the stairs."

It's been clear for years, for example, that cancers vary widely even if they look the same under a microscope: Some are slow growing, others are aggressive; some patients respond to one drug, while others are not helped at all. Yet until quite recently, the only tools available—the blunt-force mainstays of surgery, chemotherapy, and radiation—treated everyone much the same. Then, in the 1990s, powerful new computing technology allowed researchers to begin comparing the DNA of cancerous and healthy tissues and gain a look at the very blueprints of similar-looking but markedly different tumors. Here's where the advances are leading:

Toward Personalized Treatment

Louise Cooper, an elementary school teacher and endurance athlete, is one early beneficiary. Now 55, she was diagnosed in 1998 with breast cancer and received the same basic knock-it-out treatment—surgery, chemo, radiation—

prescribed for other women with similar-looking tumors. That's where Cooper's story diverged from the usual path: Her former neighbor is breast cancer expert Dennis Slamon, director of clinical and translational research at the University of California-Los Angeles Jonsson Comprehensive Cancer Center. Slamon and colleagues had discovered that in about a quarter of breast cancer patients, whose tumors were particularly aggressive, too many copies of a gene called HER2 within a cancer cell causes the overproduction of a certain protein on the cell surface that serves as a receptor for a growth-spurring substance. Cooper was tested at Slamon's suggestion and learned her tumor fit this description. "I

found out that from diagnosis to demise, it's usually about two to three years, maximum," says Cooper, an L.A. transplant who grew up in South Africa.

But Slamon and his team had developed a drug that blocks the receptors, preventing the cell from getting the fix it needs to grow. After receiving weekly infusions of Herceptin for a year, Cooper is apparently cancer free—and off to Antarctica for a multiday 250-kilometer footrace just before Thanksgiving.

You might ask why Herceptin, now a weapon of choice in women whose tumors produce too much HER2, can't be used to block all sorts of cancer from growing. As science is revealing, there are thousands

and thousands of mutated genes that contribute to cancer's uncontrolled growth and self-replication, refusal to die off, and ability to travel elsewhere in the body, lodge there, and thrive. As with HER2, a glitch might be found in some cancers in a particular organ but not others—and it may also be active in cancers in completely different organs. (HER2 is also associated with ovarian cancer.) In September, two research teams—one working on the government's massive new cancer gene-mapping initiative, the Cancer Genome Atlas project, and one on a separate private effort—described all the hundreds of possible mutations linked just to pancreatic cancer and a deadly form of brain cancer. The average pancreatic tumor, for example, included 63 mutations.

Devising treatments to combat every single mutation would be a huge task—but that may not be necessary, experts



LOUISE COOPER, 55
Her tumor called for Herceptin, which left her cancer free.



say. It seems that many of these genes cooperate together to create a cluster of distinct cellular pathways that make a cancer cell grow faster, recruit more cells, move around the body, and metastasize. "We're at Harvard Medical School looking for likely drug targets. Think of it as finding separate routes to the same destination to stop each car on the road," says Slamon. Some pathways are shared by cancers, but others are not. In a lung cancer study, for example, genes involved in cancer of the retina were not implicated in lung cancer.

Within just a few years, the sequence of an individual tumor may reveal what pathways need blocking. "We need blood tests," says M.D. Anderson's president, the American Association of Cancer Researchers. Theoretically, that means matching treatments to patients. That is, of course, not available now. Beyond that, the very effectiveness of blocking signaling pathways in certain leukemias and some solid tumors is a normal defect. Lung cancer, for example, has a cell-growth receptor EGF that is overactive but most likely involved in other pathways that work better in some patients.

Clearly, converting this massive task into perfectly tailored therapies is a huge challenge. For one thing, finding all the genes that cause cancer isn't enough. "You have to separate the wheat from the chaff—some of those changes are just noise," says Slamon. And how do the signaling pathways affect and influence each other? Even when a target is identified, it's often important and a drug made to block it, chances are the tumor has a

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described for other women with similar-looking tumors. That's where Cooper's story diverged from the usual path: Her former neighbor is breast cancer expert Dennis Slamon, director of clinical and translational research at the University of California-Los Angeles Jonsson Comprehensive Cancer Center. Slamon and colleagues had discovered that about a quarter of breast cancer patients, whose tumors are particularly aggressive, too many copies of a gene called ER2 within a cancer cell causes the overproduction of a certain protein on the cell surface that serves as a receptor for a growth-spurring substance. Cooper was tested at Slamon's suggestion and learned her tumor fit this description. "I

found out that from diagnosis to demise, it's usually about two to three years, maximum," says Cooper, an L.A. transplant who grew up in South Africa.

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Devising treatments to combat every single mutation could be a huge task—but that may not be necessary, experts



JANE TERVOORDE, 55
A carrier of the breast cancer gene, she stayed on high alert.

say. It seems that many of the aberrant genes operate together to create trouble in a smaller number of distinct cellular pathways—the molecular processes that make a cancer cell so deadly. "It has to grow faster, recruit a blood supply, and move around the body," notes Stephen Elledge, a geneticist at Harvard Medical School. These common pathways are the likely drug targets. Think of it this way: Multiple cars are taking separate routes to the same garage, and instead of trying to stop each car on the road, you block the driveway. The pathways are shared by cancers in different organs; the new lung cancer study, for example, revealed mutations also fingered in cancer of the retina and colon, among others, suggesting that drugs can be used against a panoply of tumors.

Within just a few years, the technology may be available to sequence an individual tumor's genes and find out exactly what pathways need blocking, says Raymond DuBois, provost of M.D. Anderson Cancer Center in Houston and president of the American Association for Cancer Research. Theoretically, that means drugs could be much better matched to patients. That kind of matching is only occasionally available now. Beyond Herceptin, Gleevec has proved very effective at blocking signals that encourage cell replication in certain leukemia patients with a specific chromosomal defect. Lung cancer drugs Tarceva and Iressa target the cell-growth receptor EGFR; for reasons not yet fully understood but most likely involving mutations in EGFR, they work better in some patients than in others.

Clearly, converting this mass of new genetic information into perfectly tailored therapies isn't a quick and dirty exercise. For one thing, finding all the mutations linked to a certain cancer isn't enough. "You have to separate the wheat from the chaff—some of those changes are driving the car, and others are just passengers," says Slamon. How and why are genes turned on and off? And how do the signaling pathways affect and influence each other? Even when a target is identified as important and a drug made to block it, chances are the tumor has an-

other way to reach the same end—or will find one as it continues to grow and mutate, which explains why people develop resistance to targeted therapies that work at first. "Even as patients are responding, the tumors are trying to figure out a way to escape the block," says Neil Gibson, vice president of oncology research at Pfizer Inc.

What this all suggests, many say, is that just as HIV is now managed by a drug cocktail, cancer may one day be handled as a chronic disease controlled by a changing mix of several drugs at once in sequence or combination. The race is on to ex-

pand the arsenal. According to a recent Datamonitor report, 10 new targeted drugs have entered the marketplace since 2005, and researchers are studying existing therapies to see if they're more effective together. More are in the pipeline; Genen-

tech and Roche, for example, are working on pertuzumab, which prevents the HER2 receptor from communicating with related receptors. There won't be one magic bullet, says Elledge. But "I absolutely believe we will be able to do this."

New Avenues of Exploration

While most doctors now talk in terms of managing cancer rather than curing it outright, there is one avenue of research—cancer stem cells—that might offer a permanent solution. Just as healthy adult stem cells in the body's organs produce cells for renewal and repair, the thinking goes, a small fraction of the cells in a single tumor manufacture tumor cells like little factories. Chemotherapy and radiation may seem to make a person cancer free when they actually leave the factories untouched to crank up production again later. Researchers have reported finding stem cells in leukemias and myelomas, as well as in breast, brain, pancreatic, and other tumors. Whether the cells are true stem cells or simply cells that have mutated to possess the stem-cell-like power of self-renewal is up for debate; either way, those properties are likely controlled by unique pathways that might be singled out and targeted.

It won't be easy to find and take aim at stem cells, since they're apt to look different from patient to patient, says Craig Jordan, a molecular biologist at the University of Rochester Medical Center whose lab is one of several working to develop new cancer stem cell treatments. But some drugs may already be blasting these cells.

Gleevec may work so well because it eliminates leukemia stem cells, for example. And the breast cancer drug Tykerb appears to cut the number of breast cancer stem cells.

As well as explaining how a tumor recurs, some experts theorize that stem cells may offer clues to a cancer cell's deadliest function: metastasis. Perhaps the stem

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cells themselves stealthily leave the site of the original tumor and lodge elsewhere, sometimes hiding for years before beginning to grow again. Or perhaps they are capable of producing those highly mobile cells.

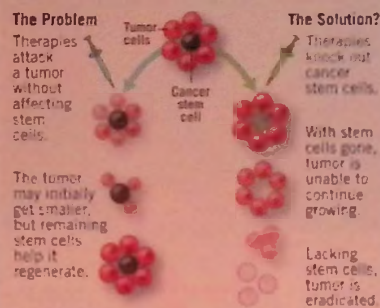
Understanding how cancer operates in the body requires looking beyond the cancer cell itself, at the tiny neighborhood of normal cells that surround it. Cancer that wants to grow and thrive has to recruit nearby cells to its cause, just as someone might borrow a cup of sugar from a neighbor. For example, the cascade of chemicals involved in the body's inflammatory response may help the cell invade healthy tissue, says David Cheresch, vice chair of pathology at Moores Cancer Center at the University of California-San Diego. And the stroma—the various nearby healthy muscle, nerve, fat, and other cells—plays its part, too. One class of drugs, including Avastin for advanced colon, lung, and breast cancer, already buys some patients extra time by targeting one part of the tumor's outreach to its neighborhood: its recruitment of blood vessels to fuel its growth.

A friendly microenvironment is also key to metastasis. Ties between a cancer cell and its neighbors are already strong by the time a primary tumor grows, says Lynn Matrisian, chair of cancer biology at Vanderbilt University. But when a cancer cell travels to a distant organ, "in my mind, there's an opportunity to intervene" before neighborly relationships are cemented, says Matrisian. Indeed, drugs used to prevent osteoporosis can also make the bone less hospitable to metastatic cells from breast, prostate, and other cancers.

Metastasis might also be foiled by immunotherapy, which prompts the body's own defenses—usually muted or silenced by cancer—into action. At the Fred Hutchinson Cancer Re-

The Cancer Stem Cell Theory

A very small percentage of cells in a tumor may be stem cells, the ones that make it so deadly. So to knock out cancer permanently, a therapy would have to attack these as well as regular tumor cells.



search Center in Seattle, researchers reported in June that an experimental treatment involving the cloning of a patient's immune cells (and injection of a superdose) apparently sent one metastatic melanoma patient into remission. The treatment didn't have the same effect on several other patients. Johns Hopkins researchers are working on therapeutic vaccines against pancreatic and breast cancers.



Like HIV, cancer may one day be managed as a chronic disease by a cocktail of several drugs.

whether the body's immune response to the earliest presence of lung cancer can be detected in the blood, much the way an HIV test searches for antibodies to the virus. Other researchers have identified a potential protein biomarker for ovarian cancer called HE4, now in use to determine how far a tumor has advanced and being studied for screening.

The challenge is not so much finding markers as it is knowing what they mean. Don Listwin, founder and chair of the Canary Foundation, a nonprofit devoted to early-detection research, points out that markers aren't very useful unless they indicate *only* cancer and can distinguish the lethal types from ones that aren't likely to progress.

Prostate-specific antigen, or PSA, for example, can indi-

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Survivors is going up because we are catching things early, before they can spread," says geneticist George Miklos, whose Sydney-based consultancy advises companies on genetics and molecular medicine.

Jane Tervooren, now 55, believes early detection saved her life. She felt for years that she was destined to get cancer; her grandmother died of ovarian cancer, and her mother did too, after surviving breast cancer. "My mother made me swear, on her deathbed, that I'd get my ovaries removed," she remembers. She did that and registered with the Family Risk Assessment Program at the Fox Chase Cancer Center in Philadelphia, where she eventually discovered she carried an in-

herited mutation in a gene, BRCA1, associated with ovarian and breast cancer. Because of her heightened risk, she received preventive medication that blocks estrogen production and had her breasts monitored frequently. Earlier this year, a mammogram detected very early-stage cancer, and after surgery, she's being treated with chemo.

Tervooren had her ovaries removed because there is no proven method to detect cancer in that organ early. Ovarian cancer, like other cancers, is screaming for some kind of advance in early detection. And the search is on for "biomarkers"—DNA, RNA, or proteins in the blood and other bodily fluids that indicate the presence or progress of disease in the body. In fact, says Lee Hartwell, president and director of Fred Hutchinson, the hope is that these markers will not only point out cancer early but also help characterize it, reveal whether it's responding to therapy, and indicate whether a recurrence is likely.

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The Best Medicine? I

It's increasingly clear that a heal

Preventing cancer is obviously the easiest—and preferable—way to save lives. So researchers are preaching what we know to do and investigating what we don't. Carel Stith is doing his part: The 63-year-old Houston lawyer is participating in a trial at M.D. Anderson Cancer Center he hopes will tell him whether, as some research has hinted, taking selenium and vitamin E supplements can stave off prostate cancer. He heard about the trial from a neighbor. "Right before that, my oldest son's godfather found out he had prostate cancer," he says. "Two weeks later, my ex-brother-in-law was diagnosed. I thought, gee, I should know a little more about this."

Vitamins and minerals are one avenue of research; in addition to the possible influence of vitamin E and selenium (for reasons unknown), there's a mounting pile of studies associating increased intake of vitamin D with a lower risk of cancer, perhaps because it plays a role in controlling the expression of genes regulating cell division. More research is needed before vitamin D is recommended

CAREL STITH, 63
Might vitamin E and selenium shield him from prostate cancer?

Cell Theory

Cells in a tumor may be like it so easily. So to truly, a therapy would have regular tumor cells.

The Solution?

Therapies knock out cancer stem cells.

With stem cells gone, tumor is unable to continue growing.

Lacking stem cells, tumor is eradicated.

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COVER STORY

cate the presence of prostate cancer. But it also leads to false alarms and detects slow-growing cancers that most likely would never have caused harm. Just this month, the Food and Drug Administration chastised the manufacturer of OvaSure for marketing—without approval—the new blood test that measures the level of six proteins associated with ovarian cancer. It's not definitive, and gynecologists worry that it will lead women to have unnecessary surgery to remove their ovaries. Canary has launched a clinical study to identify biomarkers that predict the more aggressive forms of prostate cancer and is also investigating early detection of lung, pancreatic, and ovarian cancers.

While researchers are optimistic, it's important to note that biomarkers have to be shown not just to find cancer early but to reduce mortality from the disease, says Beth Karlan, director of the Women's Cancer Research Institute at Cedars-Sinai Medical Center in Los Angeles and a member of the team that identified HE4. As history teaches, when it comes to cancer, there's good reason to temper optimism with caution. Fulfilling all this promise will take time, which won't please those who are tired of waiting. But "in 10 or 20 years," predicts M.D. Anderson's DuBois, "there will be a whole different way of detecting and treating cancer." •

The Best Medicine? Prevention

It's increasingly clear that a healthful lifestyle matters

Preventing cancer is obviously the easiest—and preferable—way to save lives. So researchers are preaching what we know to do and investigating what we don't. Carel Stith is doing his part: The 63-year-old Houston lawyer is participating in a trial at M.D. Anderson Cancer Center he hopes will tell him whether, as some research has hinted, taking selenium and vitamin E supplements can stave off prostate cancer. He heard about the trial from a neighbor. "Right before that, my oldest son's godfather found out he had prostate cancer," he says. "Two weeks later, my ex-brother-in-law was diagnosed. I thought, gee, I should know a little more about this."

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specifically for cancer prevention, but some researchers say that because of its potential to prevent other conditions, a target of 2,000 IUs a day is reasonable. Looking for clues. Beyond nutrients, recent focus has turned to-



CAREL STITH, 63
Might vitamin E and selenium shield men from prostate cancer?

ward obesity and lack of exercise as culprits. Anne McTiernan, a cancer prevention researcher at Fred Hutchinson Cancer Research Center in Seattle, is trying to identify the mechanisms that might make the obese and inactive more susceptible. Perhaps the bodywide inflammation that is elevated in the obese, for example, encourages the cancer to grow. If the mechanism is known, it would be easier to figure out whether exercising and losing weight lower risk and, if so, the

proper dose of each, she says. "If someone weighs 200 pounds now, we don't know if he or she should lose 50 pounds or 10 pounds. If they're inactive, we don't know if they should exercise for 30 minutes a day or an hour." Based on available research, the government recently recommended a weekly 150 minutes of moderate or 75 minutes of vigorous aerobic exercise, plus strength training, to help prevent diseases.

While links between household products and other environmental contaminants have been tough to tease out and neglected in research, that may be changing; environmental oncology is an increasingly vibrant field. Recently, bisphenol A, a compound found in certain hard, clear plastics, has raised widespread concerns that it may be associated with a higher risk of breast and prostate cancer. (A number of BPA-free baby bottles and other storage containers are now on the market.)

One thing is for sure: The single best evasive action is to quit smoking. The World Health Organization estimates tobacco use is responsible for 30 percent of cancer deaths in the developed world—mostly from lung but also from cancers of the head and neck, pancreas, and other organs. A pill that could save nearly a third of patients' lives would be the greatest success the field has ever seen. —K.H.

