Proceedings of the First National Cancer Conference / American Cancer Society and the National Cancer Institute of the U.S. Public Health Serivce, Federal Security Agency.

PROCEEDINGS OF THE NATIONAL CANCER CONFERENCE · 1949

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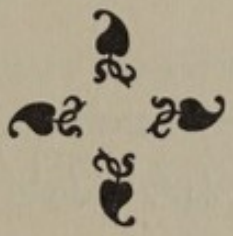
CONFERENCE



AMERICAN CANCER SOCIETY
AND
THE NATIONAL CANCER INSTITUTE
OF THE U. S. PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY

1949

Proceedings of the FIRST NATIONAL CANCER CONFERENCE

"Every chemical substance, every plant, every animal in its growth, teaches the unity of cause, the variety of appearance."

EMERSON

OBJECTIVES

I

To determine the present status of the early diagnosis and curability of cancer by site.

II

To summarize the present activities of investigators in fundamental and clinical cancer research involving etiology and epidemiology, diagnosis, newer methods of treatment, etc.

III

To appraise avenues of future efforts in clinical and investigative fields.

IV

To determine what can be done to improve the control of cancer using methods now at hand.

ADDRESS OF WELCOME

by J. R. HELLER, JR., M.D.
Director, National Cancer Institute, Bethesda, Maryland

I am happy to welcome you to the National Cancer Conference. This is the first meeting of its kind—a round-table workshop conference of clinicians, laboratory investigators, and control workers. In our working sessions, we hope to consider all major areas of the cancer problem. With every major approach to cancer represented and contributing its own special findings and experience to each of the problem areas, this conference offers a unique opportunity to advance our knowledge of cancer.

The need for better integration of laboratory work, clinical application, and control measures is almost self-evident. With every passing year, a greater proportion of our population is in the so-called cancer age group. Half a century ago, only 18 per cent of the American people—or fewer than one in five—were 45 years of age and over. Today the proportion is more than one in four. In another twenty-five years, the proportion of people in the middle and older ages will be more than one out of three.

With the progressive aging of the population, over the past fifty years, the cancer death rate has more than doubled, and the implications of this trend are profoundly disturbing to many people.

Yet there is no cause for pessimism, no reason to doubt that progress in the management of cancer will be as gratifying in the future as it has been in the past. Fifty years ago, a diagnosis of cancer was equivalent to a death warrant. Today, physicians are saving a third of their cancer patients, and the proportion seems to be steadily improving.

Research scientists, clinicians, and control people must combine forces to crack one of the central dilemmas in cancer—the fact that early cancer is relatively easy to cure but hard to discover, while late cancer is relatively easy to discover but very difficult to cure.

The simple and practical diagnostic tests that we need may possibly come from findings in basic research by someone who is

not thinking of tests at all. But it is much more probable that the contrary will be true; with the research worker made more fully aware of the needs and problem areas in the clinical and control fields, the likelihood of success would appear to be improved. In the meantime, tests that have been described in the medical literature are now under intensive investigation.

A related phase of the problem of early discovery of cancer is the effectiveness of public education. Great progress has been made in the field of adult education, in making the public conscious of the importance of early cancer symptoms. As a case-finding technique, reliance on self-discovery of symptoms may easily be confused with self-diagnosis and has obvious limitations. I understand that some attempt will be made at this Conference to evaluate educational campaigns, which may result in some specific recommendations.

The National Cancer Institute of the Public Health Service has been very glad to join with the American Cancer Society in sponsoring this Conference. Each of the panels has an exciting and important field to cover, and our hope is that the sessions will clarify many of the problems and indicate the most promising means of solving them. We can open the Conference in a confident spirit, with the knowledge that our combined experience and judgment are challenged by problems that are worthy of our best efforts.

OUTLINE OF THE CONFERENCE: ITS BACKGROUND AND ITS PURPOSES

by CHARLES S. CAMERON, M.D.
Medical and Scientific Director, American Cancer Society, New York, New York

Assuming that each of you has had an opportunity to examine with more than casual attention the program of the Conference, it is possible that some are saying "Why am I here?" and that others are noticing an unexpected unorthodoxy in the plan of the assembly and in the choice of its participants. Therefore, by explaining to you at the outset the considerations that have brought this Conference into being and by outlining the framework of its operation as we have conceived it, I hope to make you all feel that you are in the right church and even to assist you to the proper pew.

There was a time, and it is within the memory of some who are still living, when cancer was the personal concern of but two professional disciplines—the surgeon and the pathologist. Although the contributions of a few surgeons were experimental and developmental, acquisition of knowledge of cancer was, for the most part, confined to observation alone and the observation was further limited by unprecise tools, utterly inadequate for the job.

Today we can look backward on the cancer field and we can see a change—literally the change of a lifetime. For one thing, we are in a position for the first time to estimate the resources of the enemy. Many, yes, I shall say most, of the disciplines of science have seen their duty and have enlisted; equipment has been provided that will bring the cell and its contents and its behavior within the apprehension of the senses of man; money has been appropriated in insufficient but in increasing amounts, so that the annual budget for cancer has increased sixfold within the past ten years. In brief, we may say that the services of supply are being properly mobilized.

But the rapid progress that has been made now creates a new hazard. I refer to the hazard of inadequate communication. As in

military science, the importance of the services of communication is second only to the service of supply. As our numbers increase, as our equipment becomes more complex and as the territory we acquire becomes larger, the risk of losing contact among divisions grows greater.

This Conference is first and foremost a pilot project for devising a new and more effective method of assuring quick, direct communications along the expanding frontier of cancer knowledge. As such, I hope it, or a modification of it, will become a regularly scheduled event.

In addition to the mechanical difficulties of communication, which can be expected to result from the increasing complexity of our interests and operations and which jeopardizes the value of strategy, there is a personal difficulty, which grows out of the relative incapacity of the individual scientific mind to accommodate a rapidly increasing volume of data. In all aspects of human interest, the chain reaction of new facts goes on, so that today man stands before an avalanche of information, and, like the sorcerer's apprentice, is utterly unable, as an individual, to maintain his grasp of the whole. Hence, he gives his attention to a part. We are said to know more and more about less and less.

The disciplines concerned with cancer increase in number, and each discipline increases its penetrability, but at the cost of narrowing its range. Compartition is the price we pay for increasing the sum of knowledge. And yet it is probable that compartments are artificial devices set up by the mind of man in his search for the truth. They are convenient—in fact, during much of the search they are essential—but when truth is found, it is invariably found to be unconstrained and to be insusceptible of proprietorship by scientific sectarians. Basic truths are bred of many disciplines and the definitions of basic truths comes only through the synthesis of many minds and their ideas. This Conference is an attempt to find a formula favorable to that kind of synthesis. We shall, in these three days, explore together the confines of our individual disciplines, and by tapping on the barriers between us, find the spots which seem best suited to holing through.

Here is the broad pattern of the Conference (Fig. 1). It begins

this morning with the consideration, by the entire assembly, of certain biological phenomena of growth that are undoubtedly basic to the professional concerns of all of us. May I point out that the

	Friday, Feb. 25	Saturday, Feb. 26	Sunday, Feb. 27
Morning Session 9:00-12:30	Opening Session— Welcome— Dr. Nesselrode Dr. Heller Methodology of Conference Procedure — Dr. Cameron Biological Phenomena of Growth—Dr. Little	Panel Groups 1. Endocrinology—Dr. Rhoads 2. Lung—Dr. Heston 3. Head and Neck—Dr. Cope 4. Bone—Dr. Phemister 5. Lymphoblastomas—Dr. Wintrobe 6. Nervous System—Dr. Zimmerman 7. Cancer Registration—Dr. Dorn and Dr. Hammond	Panel Groups 1. Professional Education — Dr. Wangensteen 2. Cancer Services and Facilities—Dr. Branch and Dr. Sugarbaker 3. Environmental Cancer —Dr. Hueper
	Lunch	*Lunch*	*Lunch*
Afternoon Session 1:30-5:00	Panel Groups 1. Endocrinology—Dr. Rhoads 2. Lung—Dr. Ochsner 3. Head and Neck—Dr. Slaughter 4. Lymphoblastomas—Dr. Wintrobe 5. Soft-Part Tumors—Dr. Stout 6. Administration of Grants-in-Aid — Mr. Teeter	Panel Groups 1. Endocrinology—Dr. Rhoads 2. Lymphoblastomas—Dr. Wintrobe 3. Nervous System—Dr. Zimmerman 4. Epidemiology—Dr. Reed 5. Cancer Services and Facilities—Dr. Branch and Dr. Sugarbaker 6. Professional Education—Dr. Adair	1. Summaries—Panel Moderators 2. National Gastric Conference Summary—Dr. Barrett

Fig. 1.

only formal papers will be presented at this morning's session, and that all subsequent sessions, excepting, of course, the concluding summaries, will proceed according to the usual panel or conference method, based on informal and largely spontaneous

conversations. From this theme of general interest, the plan moves to specific attention to subjects that appear to have suspiciously clinical titles—titles that, I am afraid, do not convey the essential purpose of the panels, which is to bring together for free interchange of information the representatives of all disciplines that can conceivably be involved in the solution of the problem under discussion. Therefore, these so-called clinical panels will consist of three general classes of participants: first, surgeons, radiologists, pathologists, and other medical specialists, all of whom have been chosen because of their interest in clinical investigation; second, exponents of basic research, whose areas of interest appear to have actual or potential relation to clinical cancer; and third, representatives of what, for lack of a better term, I must refer to as cancer control agencies, including professional, voluntary, and official or governmental bodies. In very general terms, it is suggested that the discussion in these panels move from an initial consideration of the incidence and the effectiveness of diagnosis and treatment to the research periphery of clinical cancer where convergence with laboratory investigators may be anticipated. This is the area in the cancer spectrum where the clinician and the laboratory scientist, like the lion and the lamb, can lie down together. Finally, the implications in the foregoing conversations should be regarded by the cancer control group as the basis for discussion of possible refinements of control methods, or, to put it the other way, possible increases in the effectiveness of knowledge now at hand.

We hope that, in so far as it is compatible with reasonably complete coverage of their agenda, the clinical panels will conclude their sessions at noon on Saturday. At that point, the attention of the meeting turns again to subjects of a more general nature, namely, cancer professional educational, epidemiology, and cancer services and facilities. However, no panel will be arbitrarily expected to adjourn if, in the opinion of its members, time can be spent more profitably in continued attention to the subject under discussion.

The special designation of "State Health Officials" following the Panel subject "Cancer Services and Facilities" does not mean

that this panel is intended primarily for that group, but rather to invite their presence in a discussion of special cancer services. The Conference looks to many clinicians who are affiliated with tumor clinics and similar facilities to take part in this important panel. The final session will be held in this room at 1:30 P.M. on Sunday and will consist of brief summaries of the discussions and conclusions of each of the panels. Since the objective of the Conference is the crossing of disciplines, I sincerely hope each of you will stay to the end——when you may enjoy, in the words of Disraeli, "the ineffable glow which comes from benevolent attention to other people's business."

Part One

BIOLOGICAL PHENOMENA OF GROWTH

INTRODUCTION

by the Moderator, C. C. LITTLE, SC.D.
Director, Roscoe B. Jackson Memorial Laboratory,
Bar Harbor, Maine

The first session of this meeting has been planned with certain very definite objectives in mind.

The distinguished scientists who have kindly consented to participate in it have all made outstanding contributions to our knowledge of the great biological processes of growth and organization of which cancer is at once such a vigorous and such a recalcitrant example.

The origin, life history, control, cure, and prevention of cancer are all problems that must be answered in terms of, and in the environment of, the living organism of which the cancer is a part.

Clinical medicine deals, of necessity, with organisms in which major or minor crises of unbalance or dysfunction are present. Clinical medicine demands essentially all the abilities, energies, and loyalties of those engaged in it. Biologists, on the other hand, are or can be largely free from accountability in terms of individual Life and Death, of Health and Disease. They can invade any branch of the plant or animal kingdoms for variety of material, and they can expose that material to controlled and repeated experimentation without undue limitation or restraint.

Many of the basic principles affecting growth and organization discovered by them are of tremendous significance in the making, maintenance, and disintegration of organisms, including man.

The organizers of this Conference believe that those engaged in clinical phases of the cancer problem should be aware of some of these basic principles, both because of their inherent importance and because they are enduring stimuli to creative advance in thinking along clinical lines.

The men who will present this morning's program are the individuals best qualified to speak, concisely and with authority in their respective fields. Obviously, limitation of time precludes any

possibility of completeness of presentation. These papers will be enzymes that, we hope, will prove continuing catalytic agents in the arduous and often observation-heavy field of clinical investigation and therapy. They are not intended as a substitute for, but as a supplement to, those efforts.

I

Strain Differences in Growth Development and Disintegration

DIFFERENTIATION OF STRAINS OF GUINEA PIGS UNDER INBREEDING

by SEWALL WRIGHT, SC.D.
University of Chicago, Chicago, Illinois

I have been asked to review briefly the results of a series of experiments on the effects of inbreeding and crossbreeding in guinea pigs, conducted by the U. S. Bureau of Animal Industry from 1906 to about 1940 and a branch of this that was conducted at the University of Chicago from 1926 to 1945.

Thirty-six inbred lines were started in 1906. Matings were exclusively between brother and sister, except for one in which parent-offspring mating was followed. Twenty-three lines really got under way and were carried long enough to yield records of value. Of these, six had become extinct eleven years later, in spite of efforts to maintain them, and several others were doing poorly. At this time, all but the five most vigorous were disposed of to make room for more intensive study of these and of their crosses. It should be said that none of these was as vigorous as a control maintained from the foundation stock without the mating of even second cousins. In the later generations, side branches were disposed of in order to produce strains descended from a single mating with as many generations of brother-sister mating back of it as practicable. The records of more than 50,000 guinea pigs have been utilized.

TRADITIONAL IDEAS ABOUT INBREEDING

According to the commonest, traditional view, close inbreeding brings about an inevitable decline in all aspects of vigor.

Another idea, tracing at least to about 1750 when the first modern breeds of livestock were started, was that close inbreeding merely fixes certain characters, good, bad, or indifferent, present in the foundation stock.

THE MENDELIAN INTERPRETATION

The rediscovery of mendelian heredity in 1900 made it possible to give a rational interpretation of those traditional ideas and to define their limitations. Under mendelian heredity, the primary effect of close inbreeding is a decrease in heterozygosis as one or another allele at each locus is lost by accidents of sampling. Under brother-sister mating, about 19 per cent of the genes that are heterozygous in any generation tend to be lost in the next generation, or nearly 50 per cent lost in three generations. The end result is a high degree of *genetic* uniformity among descendants of any one mating with a dozen, or better twenty, generations of brother-sister mating back of it. As will be brought out later, however, genetic uniformity is compatible with great phenotypic variability, since accidents of development and environmental influences are, of course, not controlled in this way.

As to loss of vigor, there is reason to believe that at some, perhaps many, loci, heterozygosis is associated with greater vigor than is either homozygote. Whenever one or the other homozygote is fixed, there is a downward step that is irreversible except by mutation. If many loci are of this sort, decline in vigor becomes virtually inevitable even in the face of the most vigorous selection.

There are probably many more loci, however, at which there is merely some degree of dominance of one allele over the other. There are good theoretical reasons why the dominant phase should be that associated with higher vigor. As the random fixation of alleles increases the frequency of recessive genotypes, without change in gene frequency, close inbreeding tends to cause an average decrease in vigor on this account also. This is more easily avoidable by selection than that due to superiority of heterozygotes. Different inbred lines may be expected to differ greatly in over-all vigor and also in the particular aspects in which they show defects. Decline should end except for mutations, once genetic

uniformity has been reached. An inbred line declines toward the level of certain homozygous combinations, different in each character in each line. Figures are somewhat misleading in reference to vigor.

Great differentiation among inbred lines in relatively neutral respects, depending on which combination of genes of the foundation stock happens to be fixed, is obviously another expectation. This differentiation may indeed be expected to go far beyond that visible in the foundation stock.

Finally under the mendelian interpretation, the crossing of random inbred lines may be expected to lead to full restoration of the vigor of the foundation stock. If there has been selective elimination of lines in which the more unfavorable genes have been fixed, crossing may lead to a stock superior to the original. It may, however, require two generations to get the full effect, because of the physiological influence of the inbred mother on the first generation crossbreds.

EFFECTS ON MORTALITY AND FECUNDITY

The results of the guinea-pig experiments were in good agreement with these expectations. Let us illustrate this by considering mortality at birth during the period 1916 to 1919. Figures 1 to 7 compare the averages for the control stock B, the five larger inbred strains, and the average of the others, first crosses (CO) progeny of crossbred male with unrelated inbred female (CA), the reciprocal (AC) progeny from two unrelated crossbreds (CC), and the results from renewed inbreeding (C1, C2). Certain corrections have been made to obtain maximum comparability. The lowered viability of even the best inbreds after nine years of inbreeding, the considerable differentiation among the strains, the dependence of crossbreds on the breeding of the mother (a poor record where the mother was inbred [CO, CA] but a good one where crossbred [AC, CC, C1], and decrease on renewed inbreeding of the mother (C2) are all brought out.

Figures 1 and 2 show averages for the percentage reared of those born alive. The results differ from the preceding in the immediate improvement on cross breeding. Young guinea pigs are

FIGURES 1 to 7. The percentage of excess or defect relative to the average (A) of all inbred strains of guinea pigs in the experiments of the Bureau of Animal Industry, of a control stock (B), of the 5 largest inbred strains (2, 13, 32, 35, and 39), the average of other inbred strains (OI), first crosses (CO), the progeny of crossbred male, inbred female (CA), the reciprocal type of mating (AC), progeny from two unrelated cross breds (CC), and results of two generations of renewed inbreeding (C1, C2). These averages are for the period 1916 to 1919, beginning after 9 years of brother-sister mating.

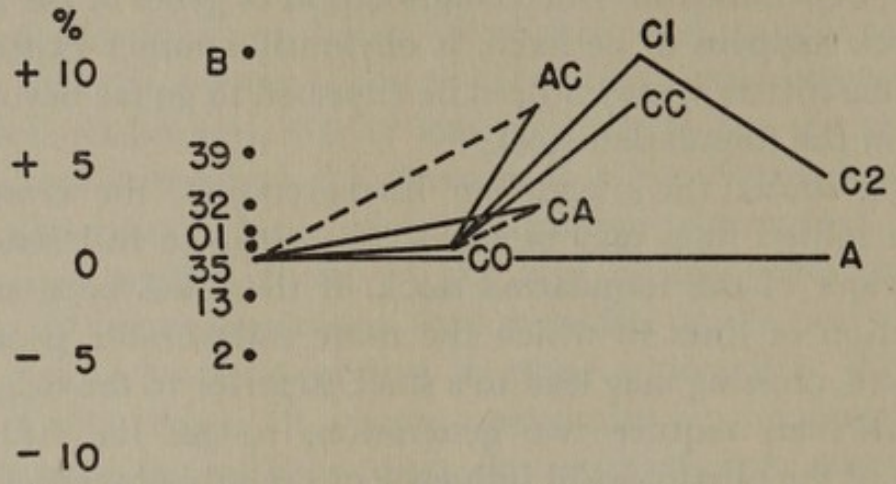


FIG. 1. Percentage born alive, corrected for effects of size of litter.

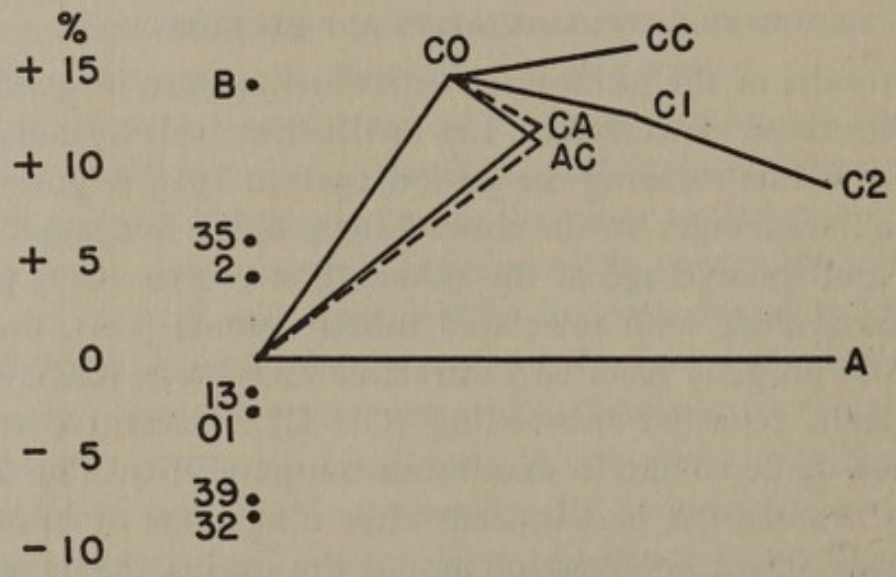


FIG. 2. Percentage raised of these born alive, corrected for effects of size of litter.

relatively independent of the mother. Another difference is in the ranking of the inbred strains, which is wholly changed. Among the twenty-two strains in an earlier period, 1910 to 1915, there was no significant correlation between the percentage born alive and the percentage reared of those born alive, although a strong tendency

toward persistence of rank in each respect demonstrated heredity.

Figure 3 gives the averages for size of litter. Again there is persistent differentiation among the inbred strains and in a new order. As expected, size of litter is almost wholly a maternal character. The very high record when the sire was inbred, dam an unrelated crossbred (AC), is probably correlated physiologically with the low record in frequency of litters from this class of matings.

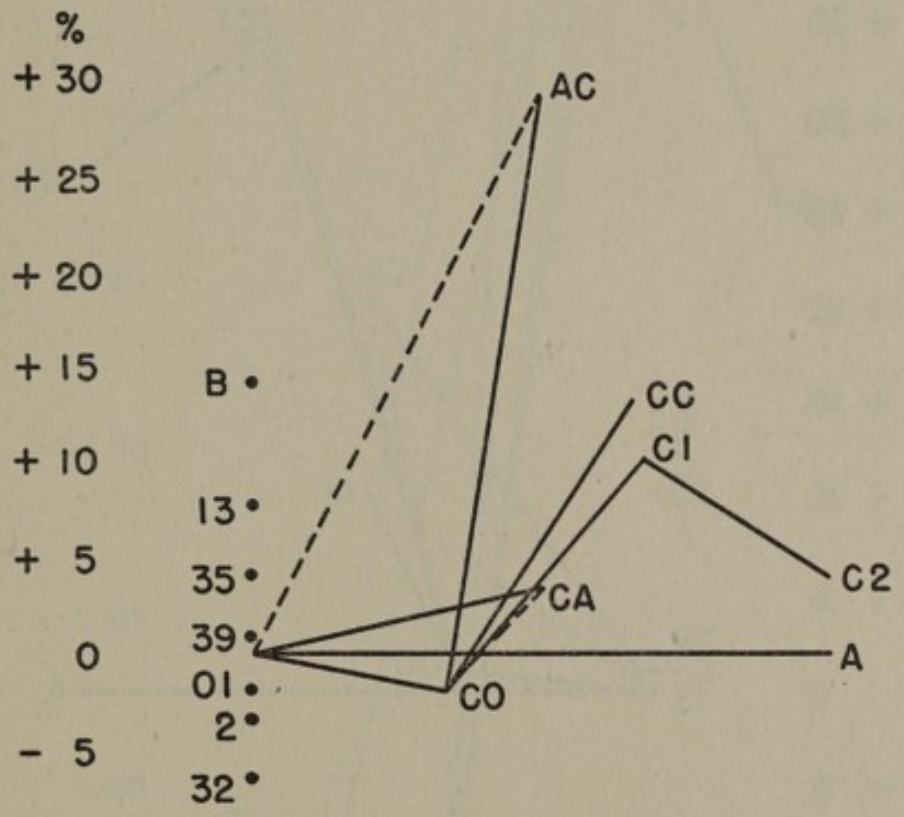


FIG. 3. Average size of litter.

Figure 4 gives data on the regularity in producing litters during this period. This is low if either sire or dam is inbred, but especially if the former. There is marked improvement when both parents are crossbred (CC, C1) in this case going far beyond the control stock.

Figure 5 shows the product of all four of these aspects of vigor, the number of young raised per mating per year. Note that when the parents are unrelated crossbreds, productivity is well above the control level. Another important point is the relatively small amount of differentiation among the inbred strains. None is at all

close to the controls. This is because of the lack of correlation among the four factors.

DIFFERENTIATION IN WEIGHT

Similar data were obtained on birth weight, weight at thirty-three days, and at later ages. The results were closely similar to

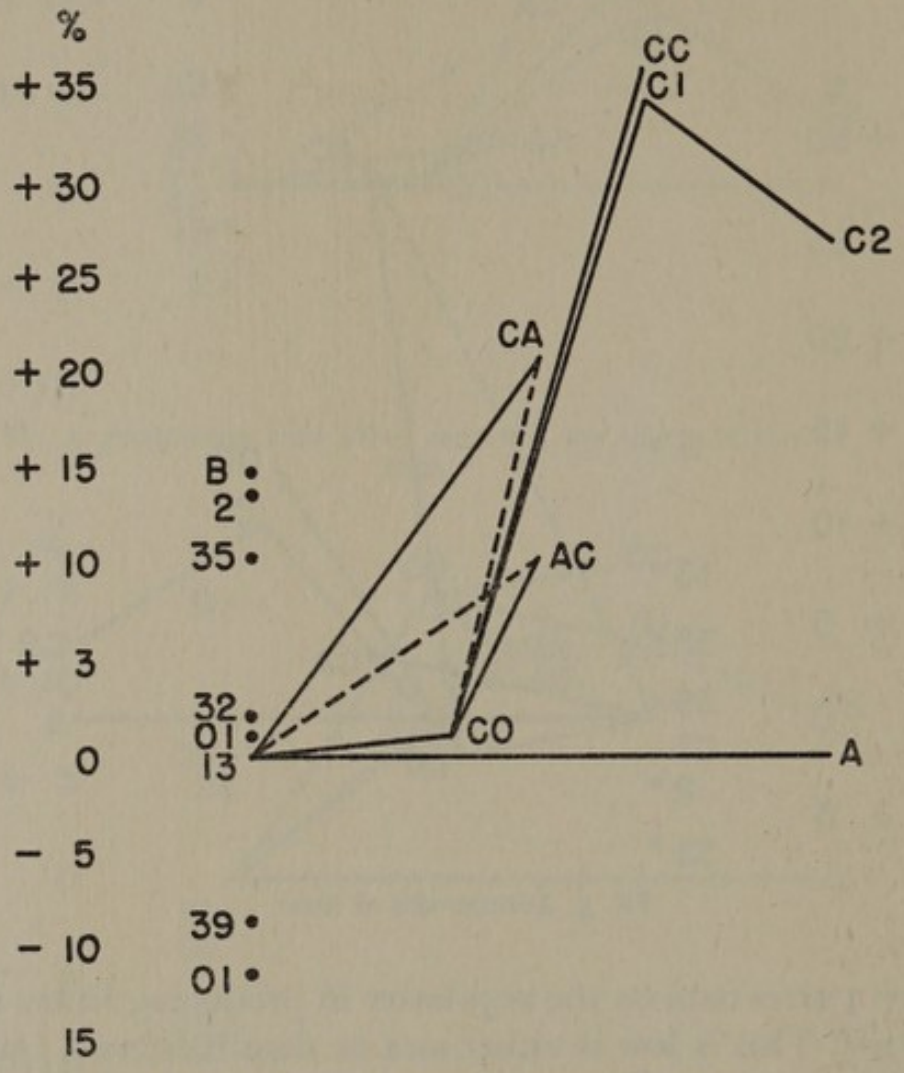


FIG. 4. Average number of litters per year.

those in mortality and fecundity. Birth weight proved to be largely dependent on the breeding of the mother, while weaning weight depended largely on that of the pig itself. There was marked differentiation among the inbred strains (some 20 per cent between extremes at birth, 30 per cent between adults). (Figs. 6, 7.)

Contrary to the situation among the preceding characters, the

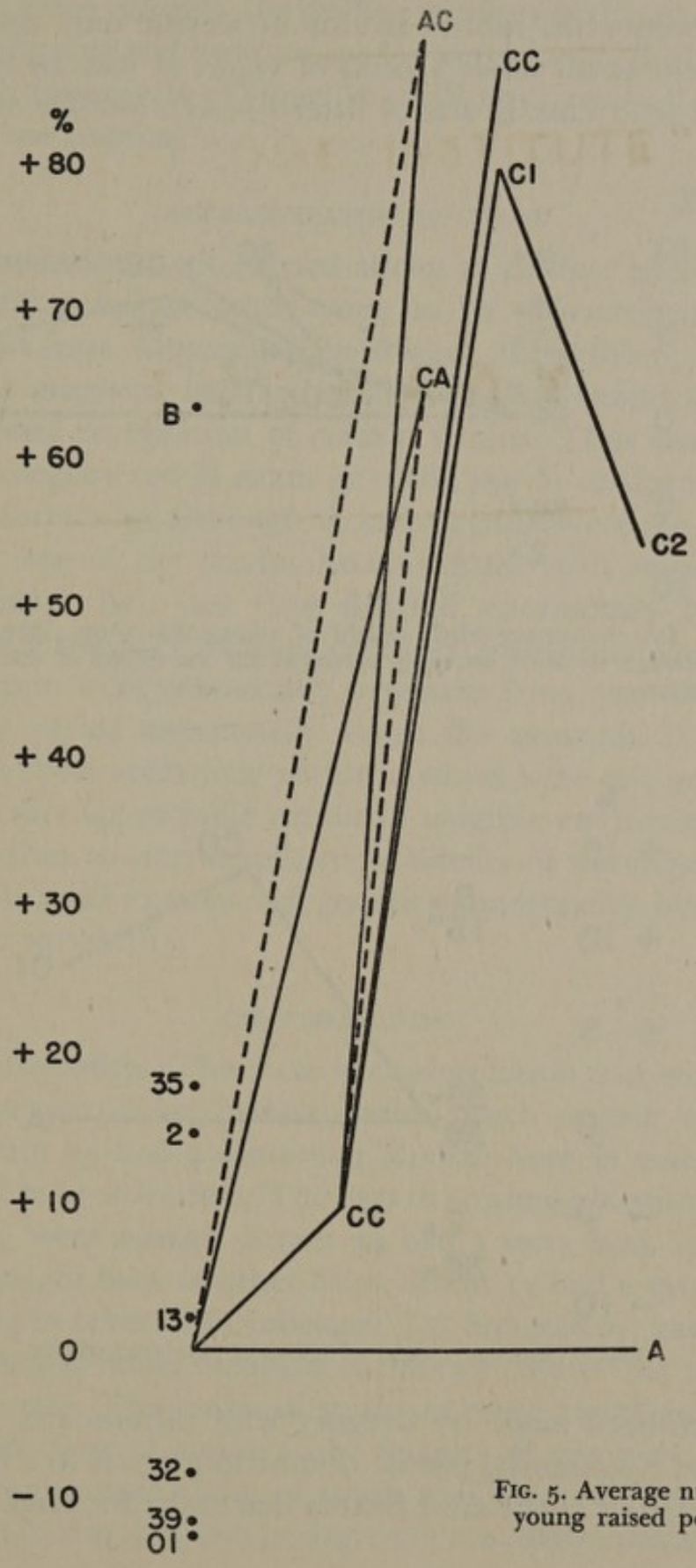


FIG. 5. Average number of young raised per year.

rank of twenty-two inbred strains in weight (any age) was not independent of all other aspects of vigor. It was, in fact, closely correlated with rank in size of litter (+ .62). Similar observations

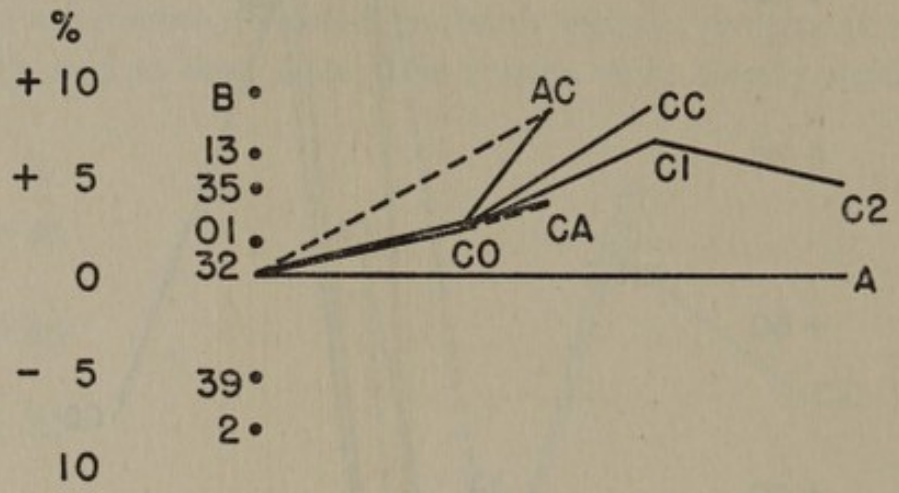


FIG. 6. Average birth weight of young that were vigorous enough to reach weaning, corrected for the effects of size of litter.

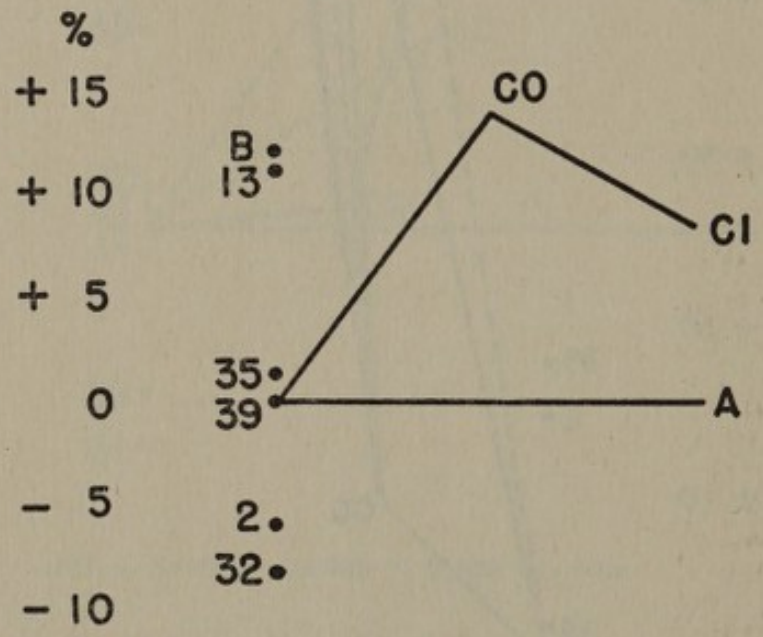


FIG. 7. Average weight of males at one year of age.

have since been made by Gregory with rabbits and MacArthur with mice. Apparently, release of multiple ova is to a large extent a manifestation of the same factors that make for large size within a species.

In all of these respects, the decline in vigor occurred during the early generations and then proceeded very slowly, if at all.

So much for aspects of vigor. We will now consider differentiation in other respects.

DIFFERENTIATION IN COLOR

The foundation stock carried alleles at six loci affecting color. Some combination or other came to be characteristic of each strain or, at least, substrain. There were, in addition, minor differences in intensity, impracticable to analyze genetically, which made for easy recognition of certain strains. Thus the uniquely intense mahogany red of strain 32 could not be confused with the light red of strain 39, although all known genes were the same.

All but one of the strains became fixed with respect to the spotting factor (ss), but they differed enormously in average amount, as a result of fixation of minor modifiers. The averages for each strain were remarkably persistent from year to year, but individuals varied enormously about the averages. It could be shown that these variations within a strain were not genetic and not due to any appreciable extent to tangible environmental factors but rather to uncontrollable accidents of development. We have here a good example of genetic homogeneity but extreme phenotypic variability.

CONFORMATION

There were subtle differences in conformation that enabled one to recognize particular inbred strains much as one recognized people. Strain 13 had a somewhat Roman nose in contrast with the pointed nose of strain 2. The eyes of strain 35 protruded, those of strain 13 were sunken. Strain 39 had a sway back in contrast with the straight back of other lines. Strain 13 had a marginal ear fold, lacking in others. My colleague, Dr. Strandskov, has analyzed measurements of bones of adults of the two strains that were most different in size. The males of strain 13 were about 30 per cent heavier than those of strain 2, the females of strain 13 about 20 per cent heavier than those of strain 2. There was not only differentiation in size, but also in degree of sex differentiation: much

in the former (11 per cent), very little in the latter (3 per cent). It is not surprising that the large strain 13 had a considerably longer body than strain 2. There was, however, no significant difference in head length, although there were some significant differences in skull proportions and the much smaller pigs of strain 2 not only had relatively, but absolutely, longer hind legs and longer upper forelegs. They were decidedly different kinds of animals.

The internal organs were also studied. The loose, stringy thyroid of strain 2 could not be confused with the compact one of strain 13. The heavy, smooth-contoured adrenal gland of strain 2

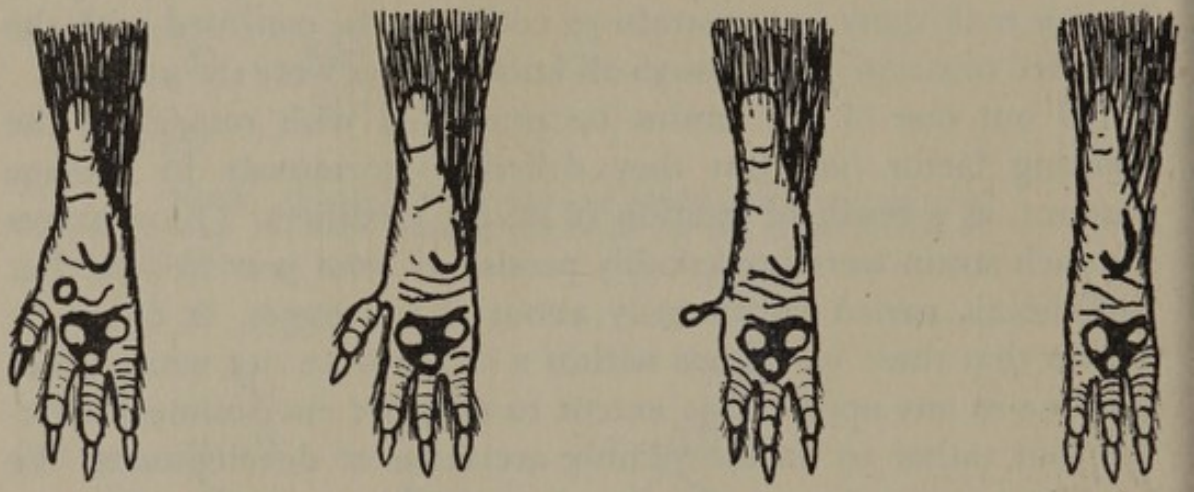


FIG. 8. Grades of development of the little toe in an inbred strain (strain 35).

contrasted with the flatter and deeply indented one of strain 13. There were also striking differences in size and shape of the spleen.

POLYDACTYLY

Another morphological character in which there was differentiation was the presence or the absence of the little toe (Fig. 8). In most strains, this toe was either wholly absent or rare. In one (strain 35), it occurred in about 31 per cent (Fig. 9). There was considerable substrain differentiation within this (0 per cent to 60 per cent), but within large substrains, it could be shown that 3 toe $\times$ 3 toe and 4 toe $\times$ 4 toe bred alike. There was again genetic homogeneity but phenotypic variability. Moreover, hidden genetic differences could be demonstrated between strains that never exhibited the character themselves. Thus crosses between strain 2

and a strain D (not of this series of inbred lines, but one in which this character was fixed) produced only three-toed young. On the other hand, crosses between strain 13, also always three-toed, and the same four-toed strain D, produced 30 per cent polydactyls. Among the latter, breeding tests revealed no difference between three-toed and four-toed.

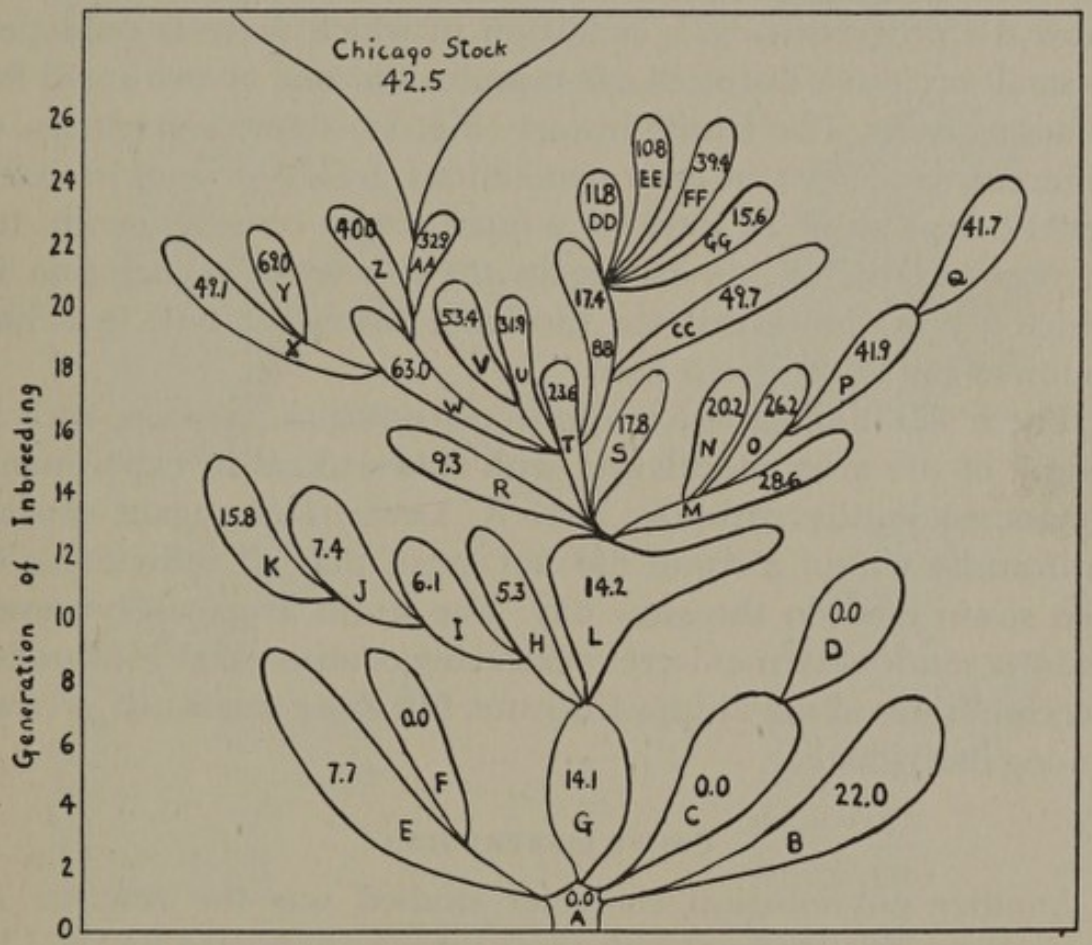


FIG. 9. Percentages of polydactyly in substrains of one of the inbred strains (strain 35).

The mode of inheritance in later generations indicated cumulative action of three or four pairs of alleles and of environmental factors (especially age of mother) with respect to the tendency to develop the little toe, a threshold at which it appears, and a second threshold beyond which there is perfect development.

OTOCEPHALY

The situation is somewhat similar with respect to a much more

extreme lethal abnormality, otocephaly (Fig. 10). These monsters were largely restricted to one inbred strain (strain 13). The lowest grades show reduction only of the mandible. Defect of the upper jaw follows. A single nostril is the external sign of an unpaired forebrain. In higher grades, there may be cyclopia, or complete absence of the eye. In the highest grade, there is no opening into the head and the only feature is a small median ear. The skulls show the progression to a condition in which there is reduction to small occipital, distorted ear capsules and one or two small flat bones in front. The family history (Fig. 11) shows segregation of different tendencies in early generations, followed by 5 per cent genetic fixation in all lines for a quarter of a century, except for the descendants of one mating in the nineteenth generation in which a mutation raised the incidence to 27 per cent in a line followed for some fifteen years.

There was differentiation also in physiological respects, such as length of life after inoculation with tuberculosis in experiments conducted jointly with Dr. Paul A. Lewis. Here again genetic uniformity within a strain did not mean that all individuals of that strain died on the same day. The strain averages, however, and the result of crosses were remarkably consistent, at least under the conditions of the Phipps Institute, but there was much overlap among individuals.

OTHER DIFFERENCES

Another physiological character studied was the reaction to transplants. Experiments with these strains, conducted by Dr. Leo Loeb, demonstrated a close approach to the results of autotransplants for grafts even from remote relatives (twelfth cousins) of the same inbred strain but rejection of grafts from other strains.

Finally, there were marked differences in behavior. The pigs of strain 35 were nervous and struggled violently when picked up. Those of strain 13 were phlegmatic and could be handled like sacks of meal.

In other studies, strains have been used as genetically homozygous material. I may refer to those of one of my students, Dr.

FIG. 10. Grades of otocephaly produced by one of the inbred strains (strain 13).

E. C. Colin, on chronic lead poisoning of male guinea pigs in which he showed with this controlled material that there is no significant genetic effect.

CONCLUSION

Summing up, these studies have presented a picture, consistent with mendelian theory, of the effects of close inbreeding in reduc-

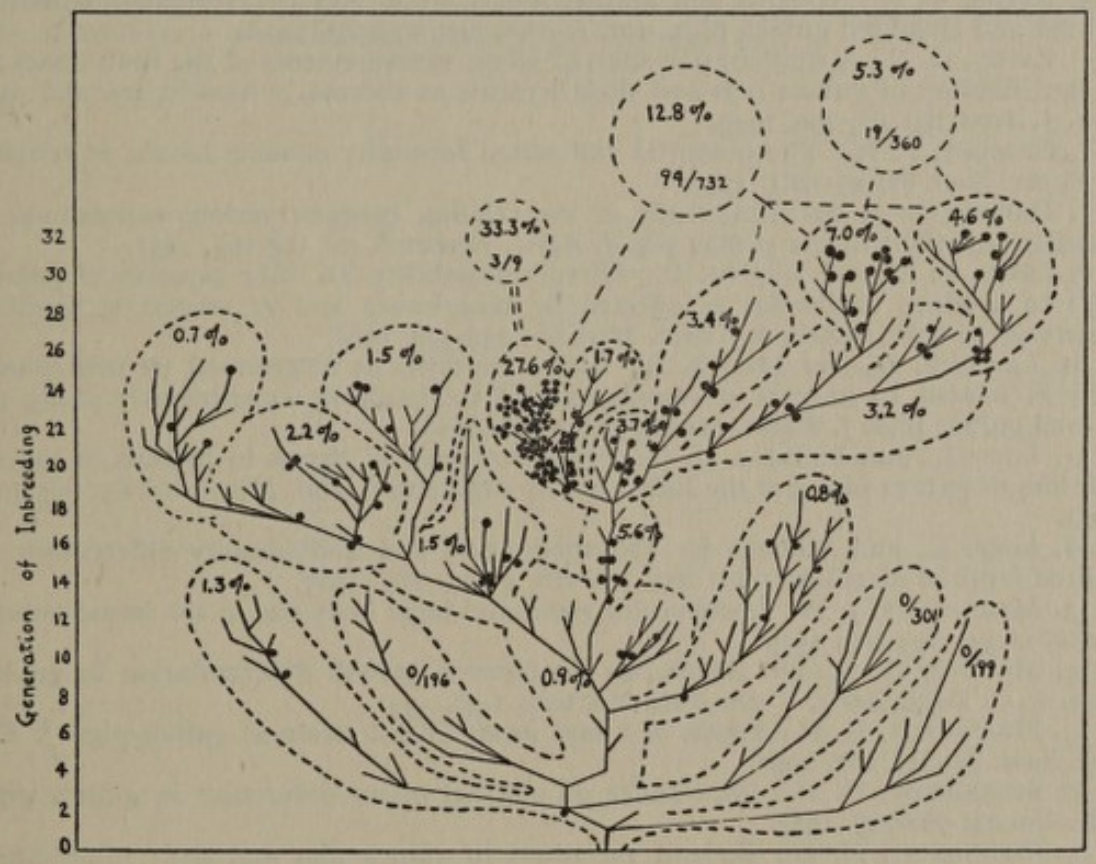


FIG. 11. The occurrence of otocephaly (black dots) in branches of strain 13.

ing vigor in all respects, on the average, during the early generations; in bringing genetic uniformity within each line and marked differentiation between lines in all respects including vigor; and recovery in vigor on crossing. The better lines could be maintained for many years, probably indefinitely, and abundantly demonstrated their value in making possible analysis of characters that could hardly have been studied at all genetically in ordinary stocks and in providing controlled material for study of special problems.

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II

Mutations

MUTATIONS IN TRANSPLANTABLE TUMORS

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It has frequently been said that the origin of cancer must be essentially mutational in nature. At some stage in the life history of an individual, a cell (or a group of adjacent cells) develops the capacity to overcome those restraints on growth that keep the multiplication of normal cells in check. As the cells multiply, further changes may occur that increase their growth potential. The result is a strain of cells with an inherited capacity for continued multiplication or autonomous growth, in other words, a cancer.

In discussing these statements, the first need is for a definition of mutation. I shall use mutation in a broad sense, but one that I think would be accepted by most geneticists. A mutation may be defined as any self-perpetuating change in an organism, a cell, or other self-reproducing unit. The change may be a minor or a major one, but it is always pictured as being discontinuous, and it must be perpetuated in succeeding generations.

With this definition in mind, let us examine various experimental results that bear on mutational processes as related to cancer. One of the early discoveries in regard to tumors was that they can be transplanted from one animal to another animal of the same species. This was first accomplished before inbred strains of laboratory animals were developed, but it is uniformly successful only within inbred strains. A number of transplantable tumors have now been carried through hundreds of transplant generations. The Jensen rat sarcoma, perhaps the oldest, has been per-

petuated by successive transplants for some forty years. Normal tissue has never been similarly perpetuated by successive transplants from host to host. It is hard to see how these facts can be interpreted in any other way than by supposing that tumor cells have acquired, by mutation, a hereditary capacity for growth not possessed by normal cells.

Once tumors have developed and been transplanted, mutational changes continue to occur in them. Perhaps the simplest demonstration of this is the almost universal observation that transplantable tumors speed up in their rate of growth during successive transplants. The change is usually gradual, but MacDowell has reported an interesting case of a tumor that up to the eighteenth transfer required some ten to twenty days to kill the host and then suddenly, with the nineteenth transfer, acquired a new virulence such that it killed in an average of about five days. For several generations prior to this increase in virulence, it was observed that the proportion of large, as compared with small, leukemic cells underwent a progressive increase, the high proportion of large cells being thereafter maintained. This transplantable tumor therefore showed both a visible, self-perpetuating change in cell type and an inherited increase in virulence.

A number of years ago, Dr. Little initiated a series of studies in the genetics of tumor transplantation, mostly carried out at the Jackson Laboratory, which, among other things, shed interesting light on the mutation process in transplantable tumors. In discussing these studies, it is necessary first to review briefly certain basic facts in regard to tumor transplantation. First, a tumor arising in an inbred strain will show 100 per cent growth in the strain of origin. Second, the tumor, with rare exceptions, will not grow at all, or will produce a small growth followed by regression, in any inbred strain other than the strain of origin. Third, the tumor will grow 100 per cent in the first-generation offspring from a cross between the susceptible and the resistant strain. Fourth, in the second hybrid generation, and in the backcross generation produced by mating the F_1 hybrid to the resistant parent, the tumor will grow progressively in some of the animals but regress or fail to grow entirely in others. The proportion of susceptible animals

in these generations ordinarily never exceeds 50 per cent and is often very much smaller than this. It has been shown by Dr. Little and his co-workers that these results are due to the existence of certain dominant genes for susceptibility that are present in the susceptible strain, and that must be present in any animals derived from this strain if the tumor is to grow progressively.

One of these genes has been shown to be identical with a gene determining a blood group in mice. Whether or not any of the other genes for tissue susceptibility are identical with blood-group genes is not known. It is likely that some of them are, but in any case, there is every reason to suppose that they are fundamentally similar to blood-group genes. They follow the same laws of inheritance, and they are strictly analogous to the blood groups in the matching of donor and host that is necessary if incompatibility is to be absent. In one respect, however, they differ from the blood-group genes in man; namely, there are more of them. The total number of genetic loci or "compatibility groups" involved in tissue compatibility in mice is something on the order of 20. Just as in any blood transfusion, however, donor and recipient would usually match for certain blood groups, so in a cross between a given pair of inbred strains, there will be matching and thus no segregation for certain loci. Hence, tests with a tumor arising in one inbred strain and innoculated into the F_2 and backcross offspring from a cross between this and a second inbred strain will ordinarily reveal some five or six genes or loci only. It would take too long here to go into the theory by which the number of genes required by a given tumor in a given cross is calculated. Suffice it to say that a large proportion of susceptible animals in the F_2 and backcross generations indicates the action of few genes—a small proportion, the action of many.

With a "young" tumor in its early transplant generations, the proportion is usually high, indicating the action of many genes. As the tumor is carried through successive hosts, however, it is found that it changes so that it grows in a higher proportion of F_2 and backcross mice. Instead of being a 5- or 6-factor tumor, it becomes a 3-, 2-, 1-, or sometimes even a zero-factor tumor. These changes are apparently sometimes gradual, sometimes sudden.

In certain cases, they have given rise to two or more recognizably different sublines of the same transplantable tumor. Thus, in an interesting experiment by Strong, an adenocarcinoma, dBrC, originating in the dba strain was tested in crosses between the dba strain and the A albino strain. In the early transplant generations, the ratio of susceptible to resistant animals indicated that six genes were required for tumor growth. Later, two substrains of the tumor were isolated, which killed a much higher proportion of F_2 and backcross mice. Calculations showed that one of these substrains required two genes, the other only one. In some way during the successive transplants, the heredity of the tumor had changed.

There are two ways of viewing this type of change or mutation in a transplantable tumor. One possibility is that the tumor, presumably originally of the same genetic makeup with respect to at least most essential genes as the tissue from which it arose, has, so to speak, changed its "tumor group." A given gene within the cells of the tumor has mutated so that it no longer produces an antigen capable of inciting resistance in host animals. This hypothesis presupposes a gene mutation as the cause of the change in the tumor transplant. The second alternative is that accompanying, or perhaps simply as an expression of, the increased virulence shown by tumors during successive transplant generations, the tumor is able to overcome the resistance in the host incited by certain of its antigens. There is reason to suppose that some of the genes and antigens concerned in resistance and susceptibility to transplants are more potent than others. Thus it is reasonable to presume that a virulent, rapidly growing tumor might be able to overcome the resistance of a host that differed from it only in the less potent genes and antigens.

This explanation also demands the occurrence of hereditary changes or mutations in the tumor, but it does not tell us whether the mutations involve genes or some other structural element of the cell. There can be no question, however, that this type of experiment proves the occurrence of mutations in transplantable tumors.

How similar is this type of mutation to the mutation or muta-

tions by which tumors originally arise? Some light is shed on this question by investigations made by Earle at the National Cancer Institute. For a long period, he carried, by the tissue-culture technique, a number of strains of subcutaneous mouse fibroblasts, some of which were treated with carcinogens while others were left untreated. These were originally entirely normal, noncancerous cells. With the passage of time, characteristic changes occurred. In certain cases, they showed a greater tendency to cohere laterally to other cells; in others, great numbers of giant cells appeared. There was also a tendency for the mitochondria to become progressively more numerous, more slender, and shorter. All of these changes were irreversible; there was no suggestion of reversion of the cells in any of the strains to their normal design.

After a certain period of culturing, cells of each strain were inoculated into mice. Whereas no normal tissue ever grows progressively when transplanted, all of these cultured strains of originally normal cells were able to grow progressively in their host. They had acquired the essential characteristics of a transplantable tumor. Here, in cells grown in vitro, we find a process of mutation at work that gives rise to a strain of tissue essentially cancerous in its characteristics.

We cannot say definitely that the types of mutation occurring in Earle's experiments and those noted in experiments with transplantable tumors are the same, but the progressive increase of virulence in each case at least suggests a fundamental similarity. It is certainly permissible to adopt as a working hypothesis the assumption that the changes in the tissues of the living organism by which tumors arise, the changes in Earle's tissue cultures, and the changes noted in transplanted tumors, are all fundamentally similar. This is an extension of the statement that the origin of cancer is essentially mutational in nature, which may be useful in guiding experimental work.

At the present time, the only value of the mutational theory of cancer is as a clue to profitable fields of research. Some clues as to where this conception may lead us can be gained from an historical analogy. The conception of the occurrence of sports or abrupt hereditary changes antedates by many years the develop-

ment of modern genetic theory. The contribution of modern genetics is the identification of these visible changes in the whole organism with changes in a self-reproducing, intracellular unit, the gene. This has greatly broadened our understanding of the mutational process and opened up a whole vast field for experimentation. If the mutational theory of cancer is to follow the same course, what we need is to acquire a knowledge and understanding of the intracellular unit or units that undergo mutational change when cancer arises. There is every reason to suppose that the gene is not the only such unit. Two cytoplasmic entities are now recognized in mammalian cells that are probably self-reproducing and capable of mutational change. These are the mitochondria, structures visible with the ordinary microscope and roughly analogous to many bacteria in their size and in certain features of their chemical composition, and the microsomes, a smaller unit visible only with the electron microscope and corresponding in size and perhaps in chemical composition to some of the viruses. Just as mutations apparent in the organism as a whole have been traced back to alterations in the gene, so there is every reason to suppose that types of mutations that occur in transplantable tumors, in cells cultured in vitro, and in the tissues of the normal individual when a tumor arises, will eventually be traced back to mutational changes in some one of the self-reproducing, intracellular entities. If this view be correct, these entities, of which genes, mitochondria, and microsomes, are the most clearly established, are of fundamental importance in cancer research.

PHOSPHATASES OF THE MITOTIC APPARATUS IN CULTURED NORMAL AND MALIGNANT MOUSE CELLS*

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The presence, in chromosomes, of an enzyme capable of hydrolyzing esters of phosphoric acid was reported in 1942 by Krugelis,[21] who used sectioned mouse testes, and by Willmer, who employed tissue cultures of chick fibroblasts. They made use of a technique described by Gomori[14, 15] and by Takamatsu, whereby alkaline-phosphatase activity is demonstrated histochemically in tissues fixed in such a manner as not to inactivate the enzyme. In brief, sections are incubated in an alkaline buffer containing organic phosphate ester and calcium ions, which precipitate the enzymatically liberated phosphate as the calcium salt; this is then visualized by conversion to dark-brown cobalt sulfide. The technique has been critically examined by Danielli[4] and concluded to give an accurate representation of the sites of alkaline-phosphatase activity if proper precautions are observed.

Further chromosome studies by Krugelis[22, 23] and by Danielli and Catcheside[5] revealed that the large salivary-gland chromosomes of the fruit fly had transverse bands of alkaline-phosphatase localization that corresponded closely to the transverse bands rich in nucleic acid,[3] earlier shown by Painter to represent locations of genes. Krugelis[23] observed phosphatase activity in mouse nuclei with the use not only of glycerophosphate but also of various nucleotides. Depolymerized desoxyribonucleic acid as substrate

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The author is indebted to Dr. Mel Green, Dr. John A. Jacquez, and Dr. Eugene Roberts for helpful suggestions.

gave reactions chiefly in the nucleus and to a slight extent in the cytoplasm, while the reverse was true with ribonucleic acid. Dempsey and Deane found an especially strong reaction in the chromosomes of mouse duodenum when glucose-1-phosphate was used as substrate.

Willmer employed sodium glycerophosphate as the substrate with his alcohol-fixed tissue cultures. He described the presence of active enzyme in the nucleoli of resting cells and in the chromosomes, inner mass of cytoplasm, centrioles sometimes, and cell membranes of dividing cells.

We have repeated Willmer's observations on cultured cells and extended them with other substrates.

The technical details will be stated only briefly here. Cultures of embryonic Akm mouse skin and two mouse tumors, sarcoma T241 and lung carcinoma MA387 (now largely sarcomatous), were planted on coverslips inserted into flattened roller tubes with the customary plasma clot and fluid medium of salt solution, embryo extract, and serum. After one or two days incubation, the cultures were washed in saline, fixed in chilled 80 per cent alcohol or a combination of alcohol, pyridine, and formalin suggested by Danielli,[4] held at −20°C. overnight, and then incubated in the medium for the demonstration of phosphatase activity. This medium contained sodium barbital, 2.1 mM; calcium chloride, 15 mM; organic phosphate, 0.1 or less to 20 mM; and occasional activators or inhibitors. The *p*H was adjusted as desired, optimally to *p*H 9. The preparations were incubated for one hour to one day and then passed through the usual calcium chloride, cobalt chloride, and ammonium sulfide solutions to make visible the sites of phosphatase activity.

To Willmer's observations of positive reactions in chromosomes generally and in centrioles, we are able to add the observations of alkaline-phosphatase activity in spindle fibers (which Willmer showed in his drawings but did not mention in the text) and a greater concentration of enzyme in the centromere region than in the rest of the chromosome. Substrate hydrolysis occurred in these locations with sodium glycerophosphate, yeast adenylic acid from

Description of Figures

All figures are photographs of mouse cells in tissue culture prepared for alkaline-phosphatase demonstration, taken under oil immersion. (About × 1600.)

Fig. 1. Embryonic mouse skin. Incubated in 13.3 mM glycerophosphate for twenty-one hours. An early prophase with heavily darkened chromosomes and nucleoli.

Fig. 2. Same data as Fig. 1. Mitotic metaphase, side view, showing darkened spindle fibers.

Fig. 3. Tumor MA387. Incubated in 0.5 mM ATP for eighteen hours. Metaphase, side view, with spindle separated from cytoplasm by pressure on coverslip.

Fig. 4. Embryonic mouse skin. Fixed in 80 per cent alcohol at —20°C. Incubated for one hour in 2 mM ATP. Early anaphase side view, with darkening most noticeable on centrioles and centromeres, less so on rest of chromosomes and spindle.

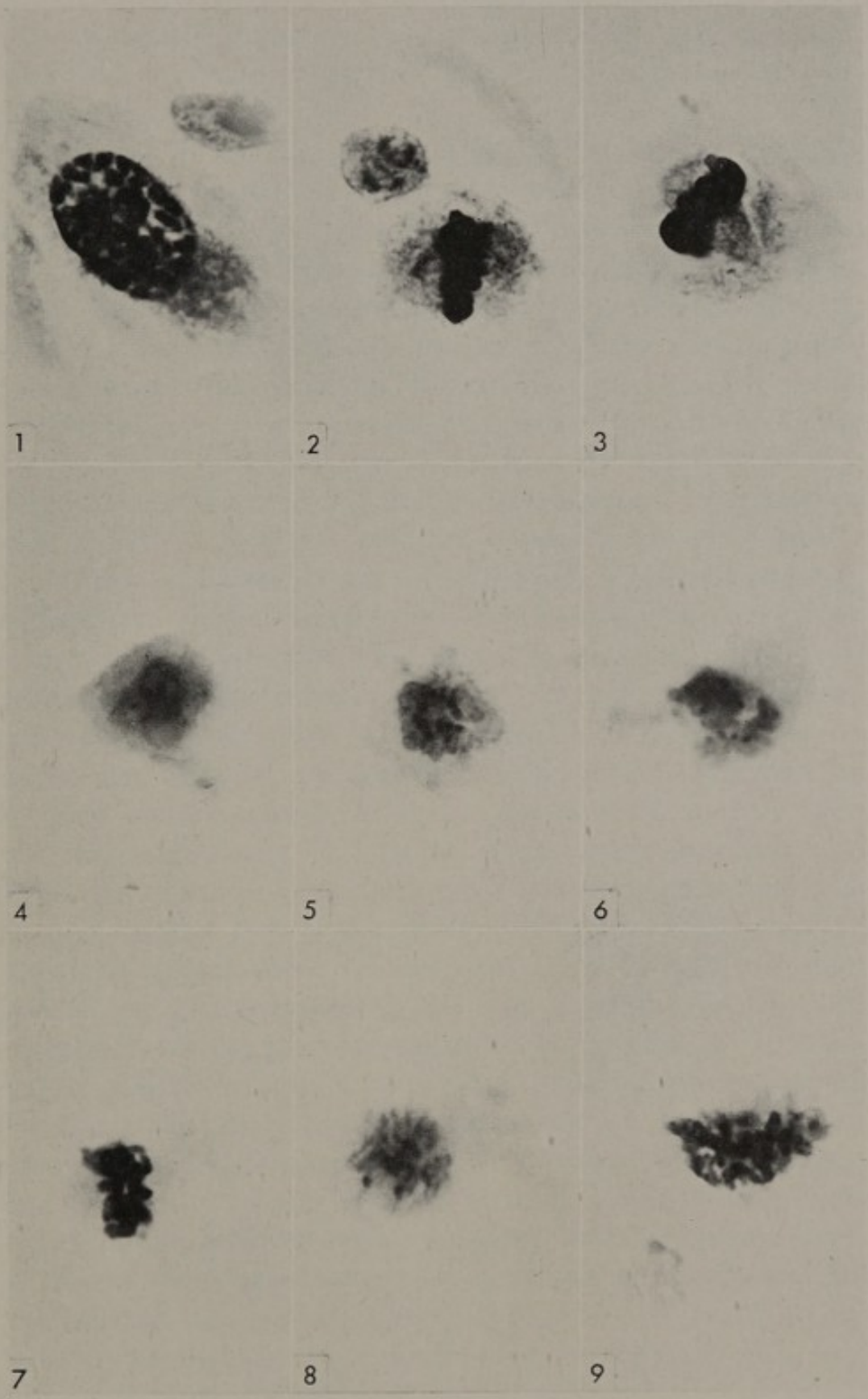
Fig. 5. Tumor T241. Fixed in 80 per cent alcohol. Incubated twenty hours in 20 mM glycerophosphate. Metaphase, polar view, showing inner ring of darkened centromeres within ring of gray chromosomes.

Fig. 6. Embryonic mouse skin. Fixed in cold 80 per cent alcohol. Incubated eighteen and a half hours in 0.2 mM ATP. Mitosis showing chromosomes with heavier stain at one end (centromere region).

Fig. 7. Embryonic mouse skin. Fixed in cold 80 per cent alcohol. Incubated four hours in 6 mM yeast adenylic acid. Metaphase side view, with chromosomes heavily stained.

Fig. 8. Tumor T241. Fixed in cold alcohol + pyridine + formalin. Incubated twenty-one and a quarter hours in 1 mM yeast adenylic acid. Late prophase, weakly stained.

Fig. 9. Tumor T241. Fixed in cold alcohol + pyridine + formalin. Incubated twenty-one and a quarter hours in 0.1 mM ATP. Prometaphase, with chromosomes heavily stained.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four sources, muscle adenylic acid, and adenosinetriphosphate from five sources.* Figures 1 to 6 are photomicrographs of cultured normal and malignant cells showing phosphatase activity with various substrates.

The splitting of the metabolically important substance adenosinetriphosphate on the centrioles, spindle, centromeres, and chromosomes might tempt one into hypotheses concerning the presence of specific ATPases or adenylpyrophosphatases in these locations. Demonstration here of enzymes capable of splitting energy-rich phosphate bonds and making available their approximately 11,000 calories per mole (Lipmann), rather than the 3000 calories per mole from ester phosphate hydrolysis, would be highly satisfactory to cell physiologists. With reference to the close relation between myosin and adenosinetriphosphatase,[8] the analogy between spindle fibers and myofibrils[37] could be more closely drawn. It appears, however, that the hypothesis of a specific adenylpyrophosphatase rather than less specific alkaline phosphomonoesterase present on the mitotic apparatus in cultured mouse cells might be subject to some doubt. The announcement by Glick and Fischer[12] of the histochemical demonstration of ATPase activity was questioned by Moog and Steinbach[31] on the basis that alkaline phosphatase could catalyze the hydrolysis of ATP, as had been claimed by Liebknecht with bone phosphatase. Schmidt and Thannhauser had found that their purified intestinal phosphatase could split off the two acid-hydrolyzable phosphate groups of adenylpyrophosphate more readily than it could split the ester linkage. Moog and Steinbach[31] felt that a valid histochemical demonstration of adenylpyrophosphatase would depend on a difference in localization from sites of alkaline phosphomonoesterase activity or on a greater rate of calcium phosphate deposition in a given region in the presence of ATP than with glycerophosphate. To this, Glick[11] added that it would be well to utilize differences in properties of adenosinetriphosphatase and phosphomonoesterase. Gomori[16] has stated recently that probably only one enzyme was responsible for the histochemical phosphatase reaction ob-

* The author is indebted to Dr. G. A. LePage of the McArdle Laboratory for a sample of ATP and to Dr. Paul Roll of the Sloan-Kettering Institute for samples of ATP and yeast adenylic acid.

tained with some eighteen different substrates, including ATP, and he has added that evidence for the presence of different enzymes must depend on definite differences in the picture of enzyme localization under conditions changed with respect to substrate, *p*H, or inhibitors and activators.

In our cultured mouse cells, there appeared to be identity of localization of calcium phosphate deposition, no matter which substrate was used.

However, there were differences in the concentrations of these compounds needed to achieve a given degree of blackening under otherwise similar conditions. In order of decreasing effectiveness, the substrates were muscle adenylic acid, ATP, yeast adenylic acid, and glycerophosphate, with a difference of the order of five-fold between successive compounds. There were some interesting differences between normal cells and sarcoma cells in this respect: normal-cell chromosomes seemed to split yeast adenylic acid at lower concentrations with somewhat greater readiness than did the sarcoma-cell chromosomes. This difference was not present or was not so apparent with muscle adenylic acid or adenosinetriphosphate, which have the same fundamental nucleotide skeleton and differ from most yeast adenylic acids in having the phosphate attached to the fifth carbon of the ribose rather than the second or third. (See Figs. 7 to 9.)

Differentiation of hypothetical adenylpyrophosphatase activity from alkaline phosphatase activity by means of *p*H proved fruitless. With *p*H optima of about 9 for the splitting of both, the ATP hydrolysis in the fixed cells was detected down to about *p*H 8.1 and the glycerophosphate hydrolysis to about *p*H 8.3.

Magnesium, which is held to activate alkaline phosphatase optimally at about 10 mM[1, 2, 17] and to inhibit adenosinetriphosphatase, at least above certain low concentrations,[7, 26, 38] progressively inhibited the splitting of ATP in the mouse cells at and above 10 mM with calcium constant at 15 mM. Magnesium seemed somewhat to activate the hydrolysis of glycerophosphate and adenylic acids at 10 mM but depressed their splitting at higher concentrations, especially when the ratio of magnesium to calcium concentration had reached 4:1. This evidence would seem to be

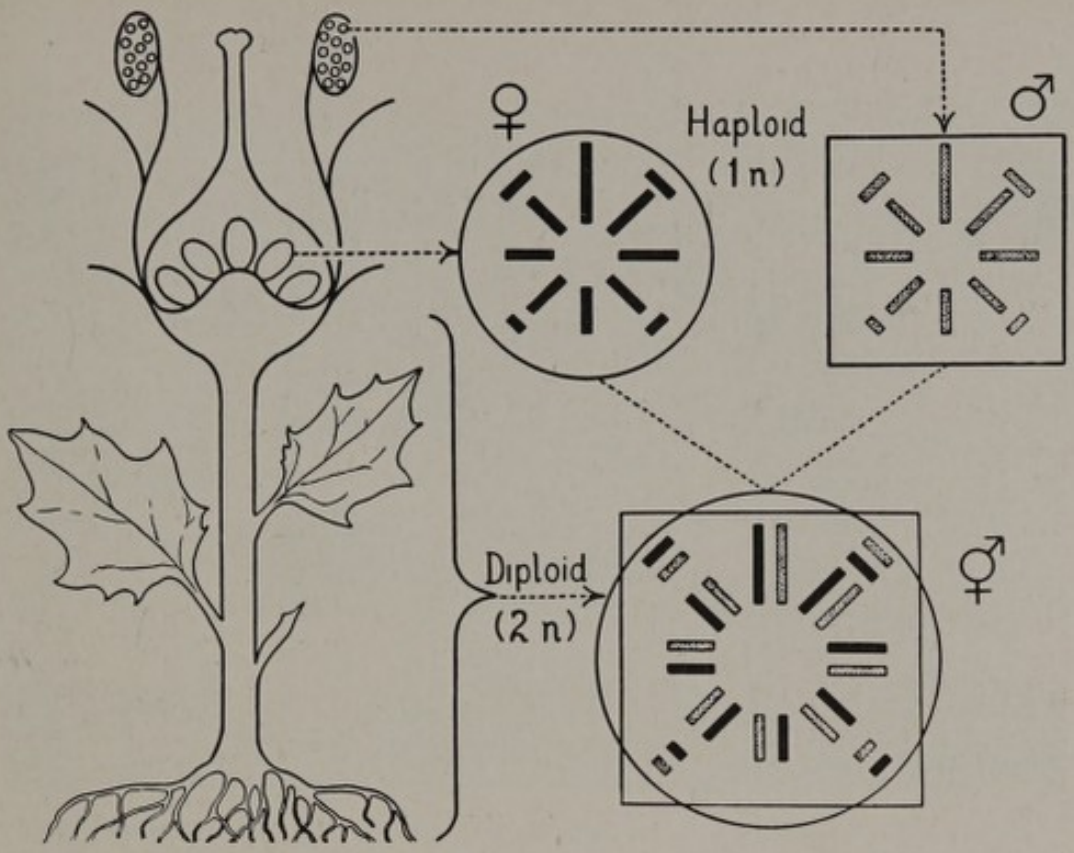

FIG. 1. Diagram to show reproductive cycle in the Jimson weed *(Datura stramonium)*. Models of chromosomes from the egg cells (♀) are represented in solid black, those from the sperm cells (♂) derived from the pollen are stippled.

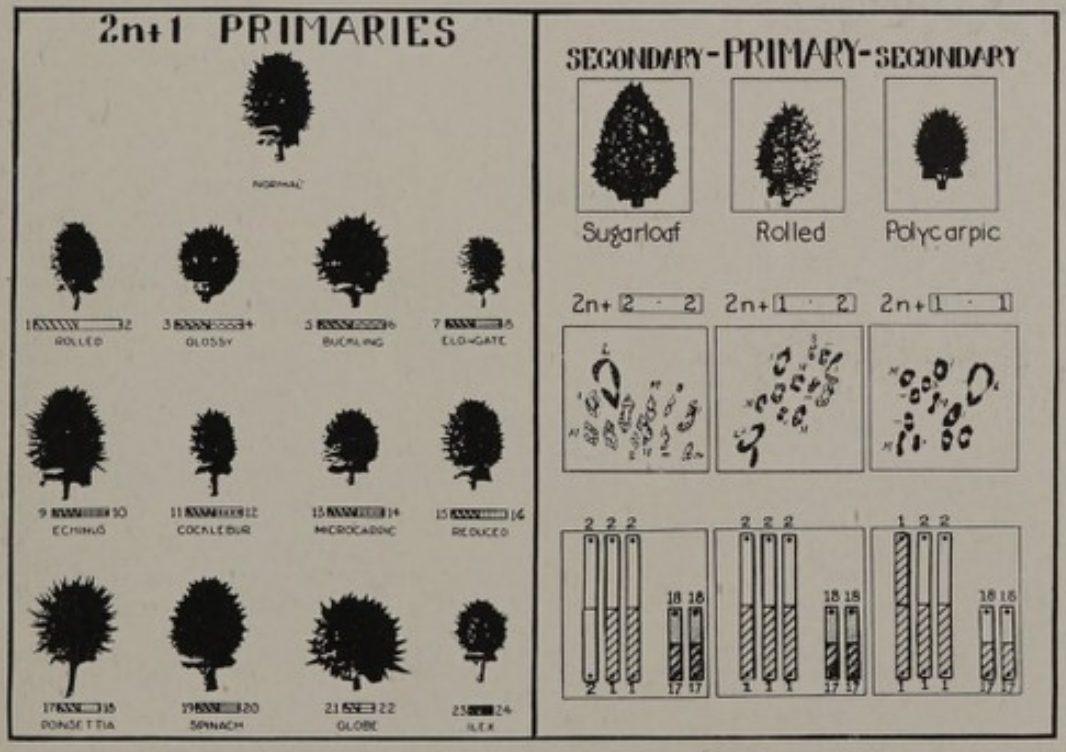

FIG. 2. Fruit of a normal $2n$ *Datura* and below fruits of the twelve primary $2n + 1$ chromosomal types, each with an extra unmodified chromosome represented by models with numbered ends. Before their chromosome nature was known, these mutant types were distinguished by pet names given below each fruit.

FIG. 3. In center, fruit of a primary $2n + 1.2$ type. On either side, fruits of the complementary secondaries ($2n + 1.1$ and $2n + 2.2$) in which the extra chromosome is composed of one half chromosome doubled. Below each fruit are shown drawings and models of their chromosomes.

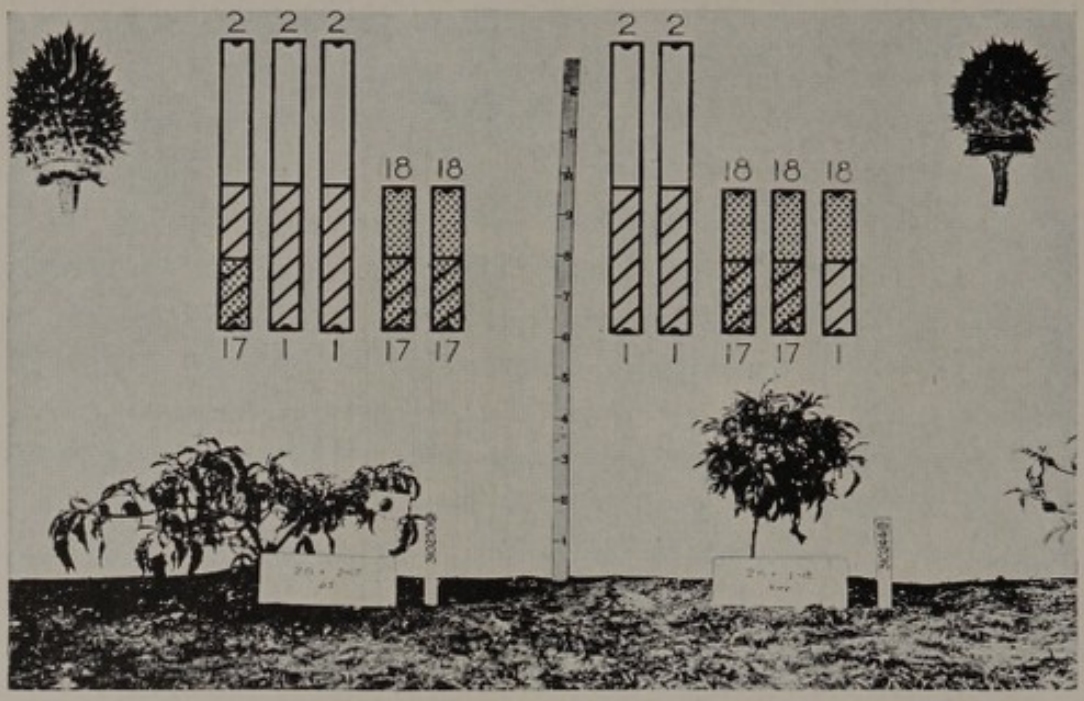

FIG. 4. Two tertiary $2n + 1$ types in which the extra chromosome is composed of parts of two different chromosomes, together with models of the chromosomes involved.

FIG. 5. On the left, green-stemmed shoot from a bud sport in which a chromosome fragment containing the dominant gene for purple stem was eliminated from a plant hybrid for purple and green stem.

FIG. 6. Normal ($2n$) plant of *Datura* with tetraploid ($4n$) branch on left. Such $4n$ branches can be induced by treatment with the alkaloid colchicine.

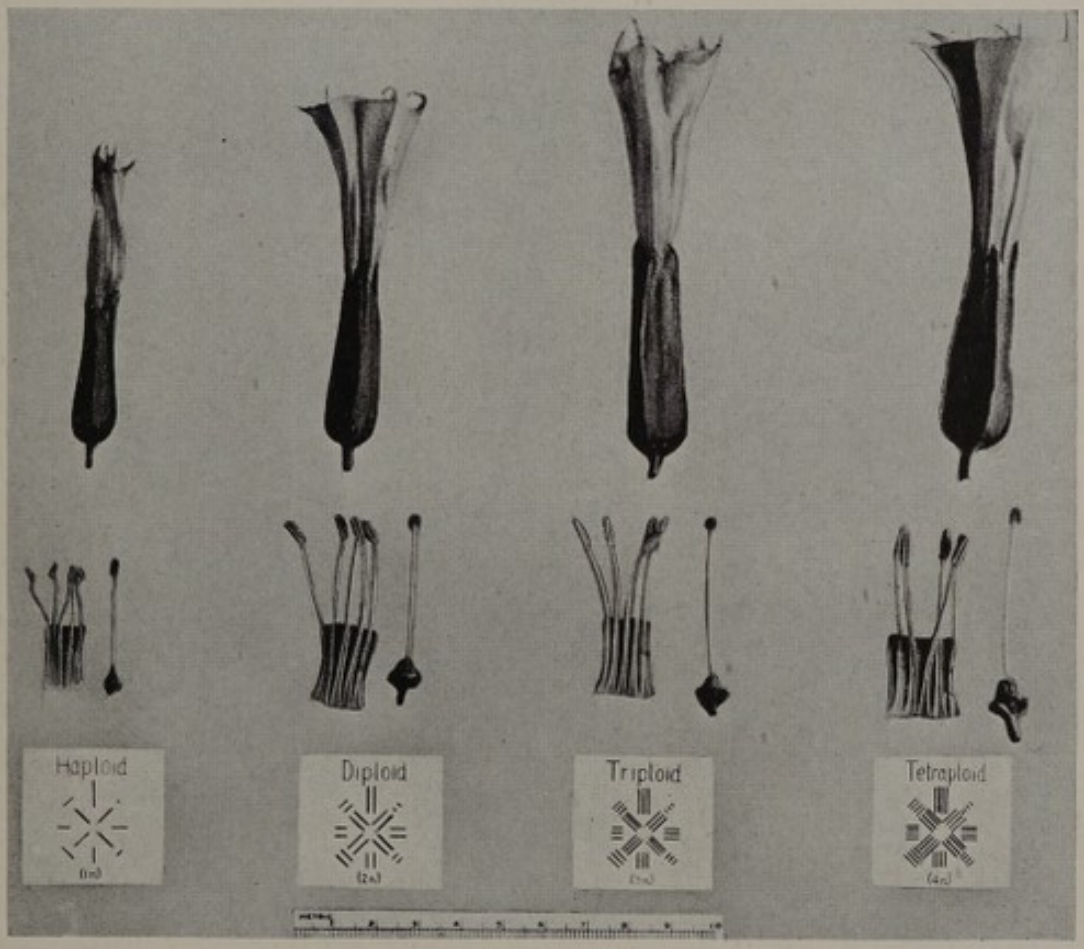

FIG. 7. Flowers of $1n$, $2n$, $3n$, and $4n$ *D. stramonium* showing increase in size with increase in chromosome number.

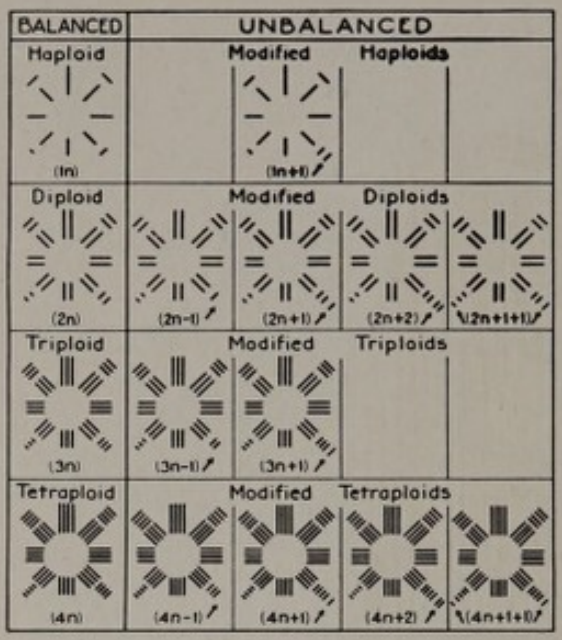

FIG. 8. Diagram with models to show some of the balanced and unbalanced chromosomal types secured in *D. stramonium*.

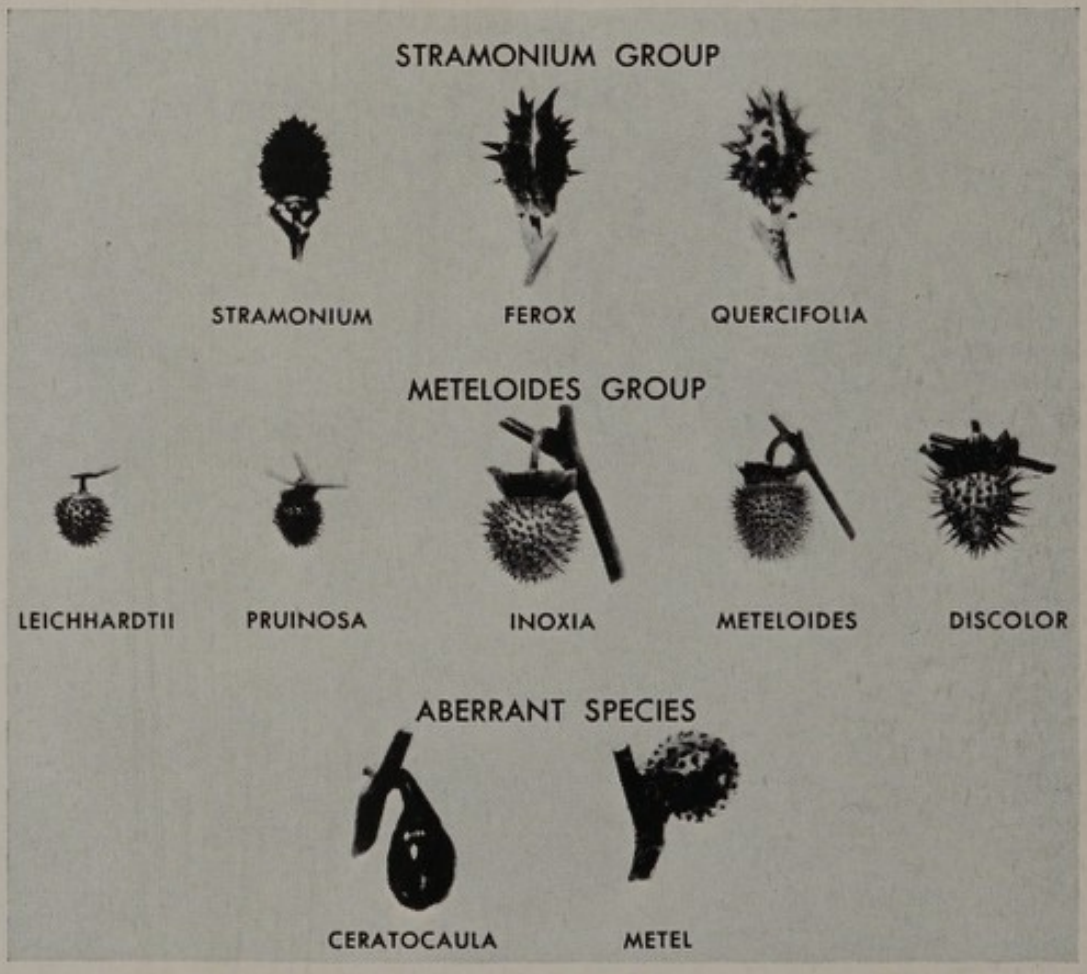

FIG. 9. Fruits of the ten herbaceous species of *Datura* cultivated at the Smith College Genetics Experiment Station.

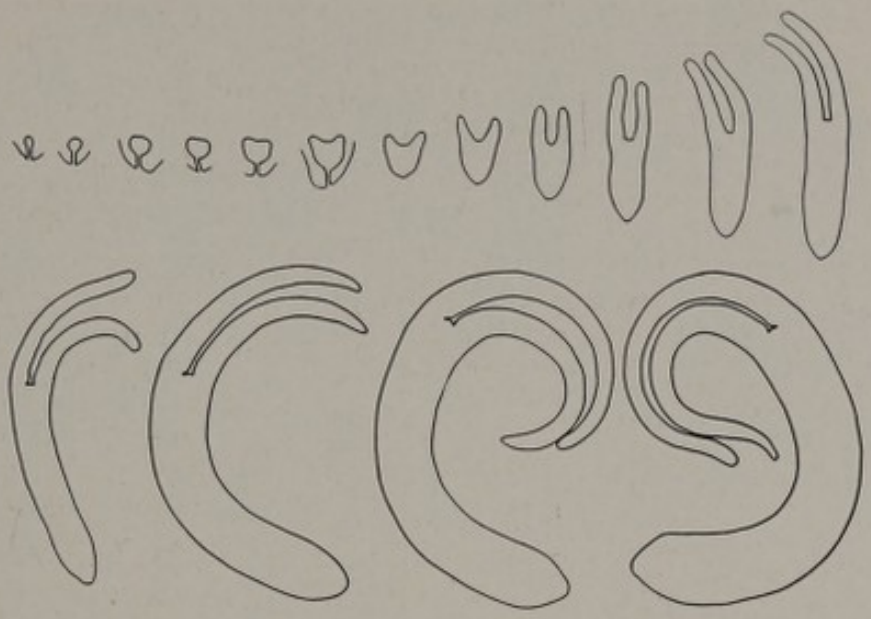

FIG. 10. Stages in development of embryos of *D. stramonium* from about a week old with diameter of ± 0.1 mm. at upper left to month old mature embryos at lower right.

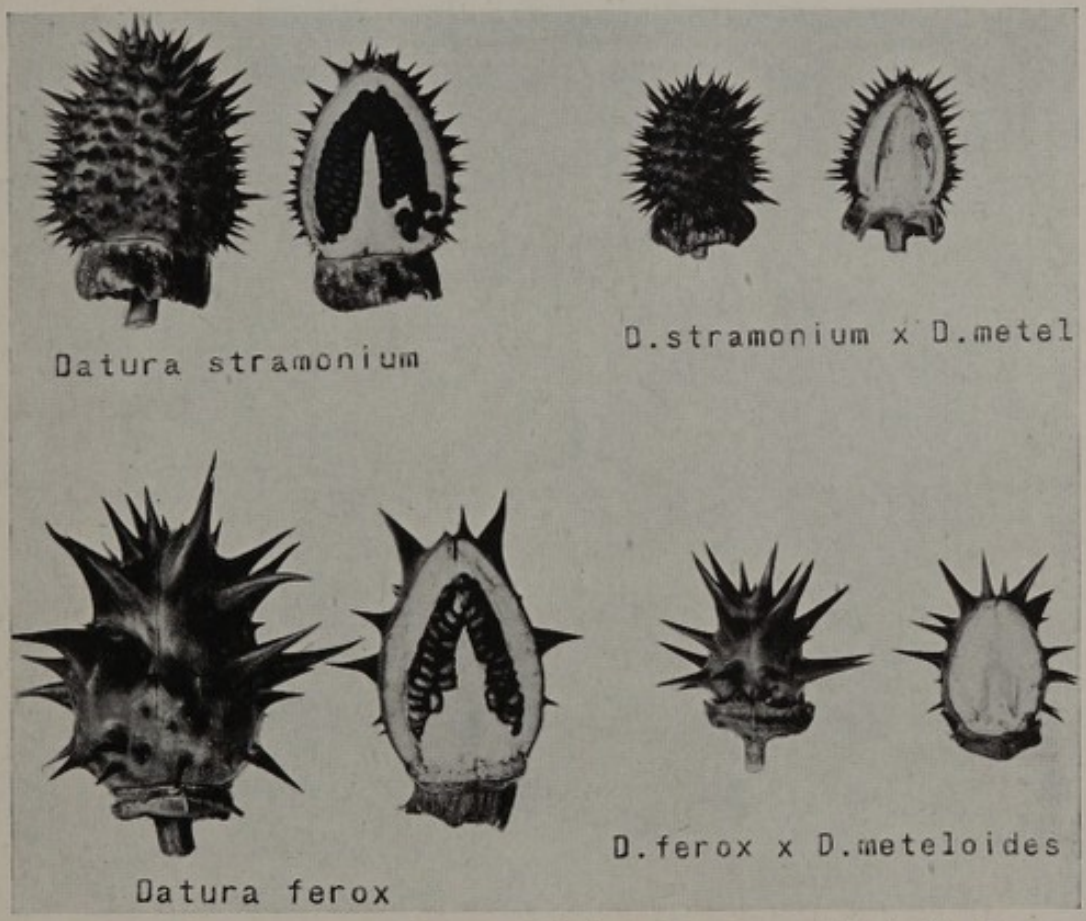

FIG. 11. Below, intact and opened fruits of *D. ferox* with similar fruits from the cross *D. ferox* × *D. meteloides*. Above, fruits of *D. stramonium* and fruits from the cross *D. stramonium* × *D. metel*.

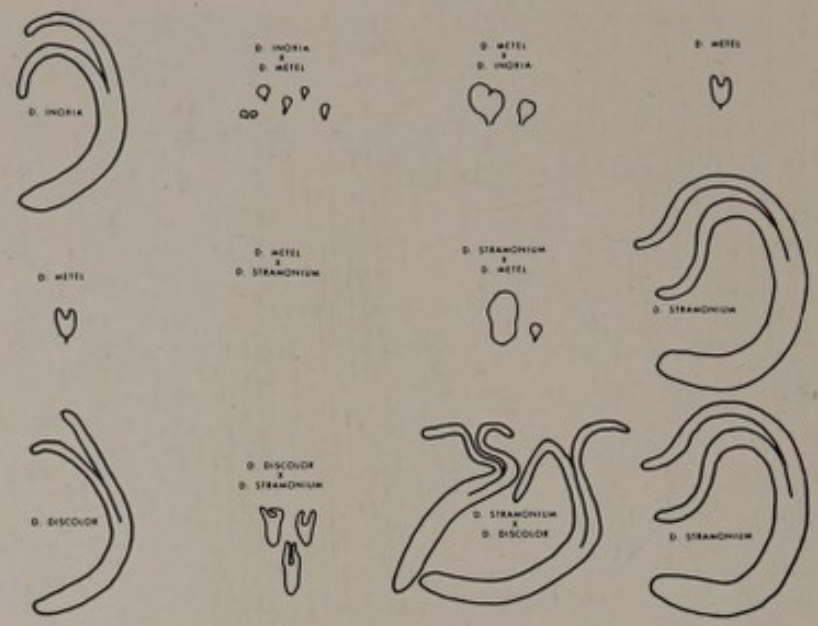

FIG. 12. Normal embryos of several species of *Datura* and hybrid embryos from crosses between them.

INTERSPECIFIC CROSSES IN DATURA

♀ \ ♂	STRAM.	QUERCI.	FEROX	PRUINOSA	LEICH.	DISCOLOR	CERATO.	METELOID.	METEL	INNOXIA
STRAMONIUM	£	X	X	—	[ED]	X	[ED]	[ED]	[ED]	[ED]
QUERCIFOLIA	X	£	X	[ED]	ED	X	[ED]	[ED]	[ED]	ED
FEROX	X	X	£	ED	[ED]	X	[ED]	E	[ED]	ED
PRUINOSA	ED	[ED]	[ED]	£	[ED]	ED	[ED]	ED	E	[ED]
LEICHHARDTII	X	X	X	X	£	X	[ED]	X	[ED]	X
DISCOLOR	[ED]	ED	—	—	—	£	[ED]	[ED]	[ED]	E
CERATOCAULA	—	—	—	—	—	ED	£	E	[ED]	ED
METELOIDES	—	—	—	—	—	E	[ED]	£	ED	ED
METEL	—	—	—	—	—	E	ED	X SP.	£	X SP.
INNOXIA	[ED]	—	—	—	—	[ED]	[ED]	X	[ED]	£

FIG. 13. Chart to show fertile hybrid seed (×) and dissected hybrid embryos (E D) secured from cross pollinations among the ten species of *Datura*. E alone indicates embryos determined in microscopic sections but too small to be seen with dissecting microscope. [E D] indicates hybrids that have grown to maturity in soil. Females (♀) are listed in left vertical column, males (♂) in upper horizontal row. The pound sterling sign indicates selfed seed. × SP indicates an extremely rare (sporadic) seed.

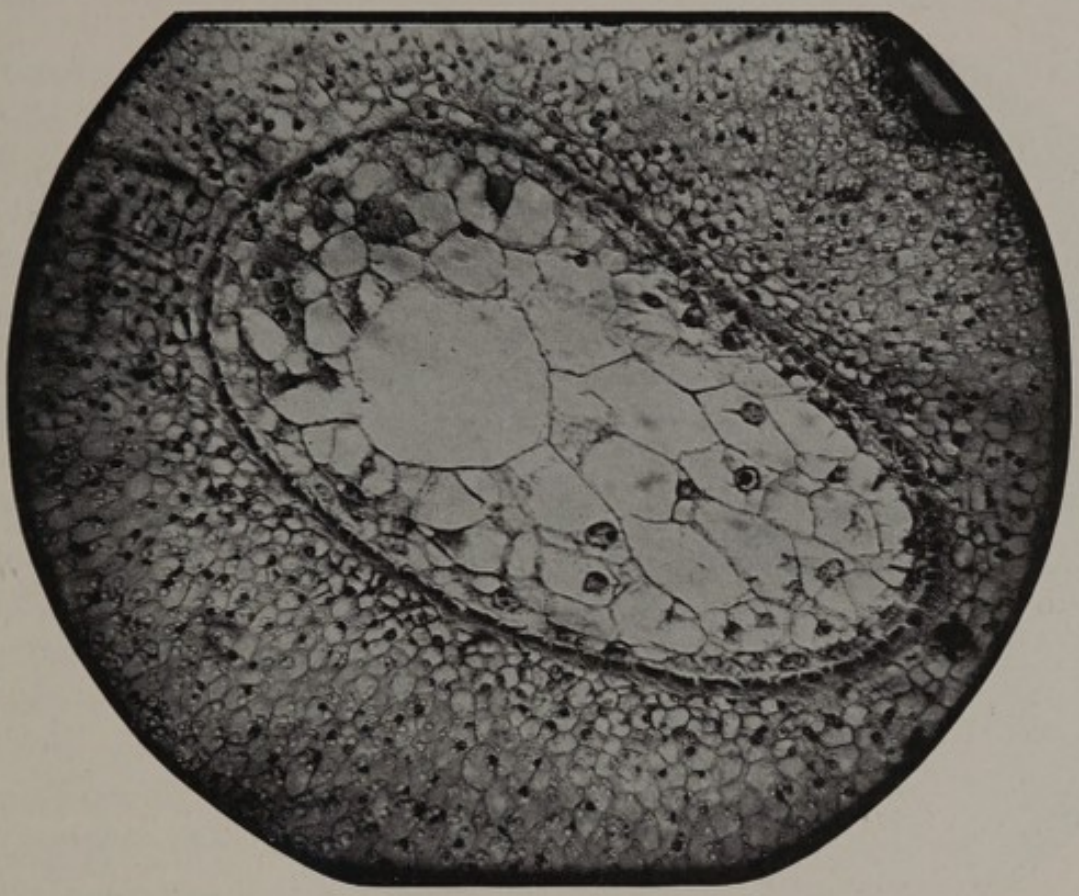

FIG. 14. Section of young normal ovule. Heart-stage embryo had dropped out from open space near center. Note single layer of dark-staining nurse cells surrounding the central tissue.

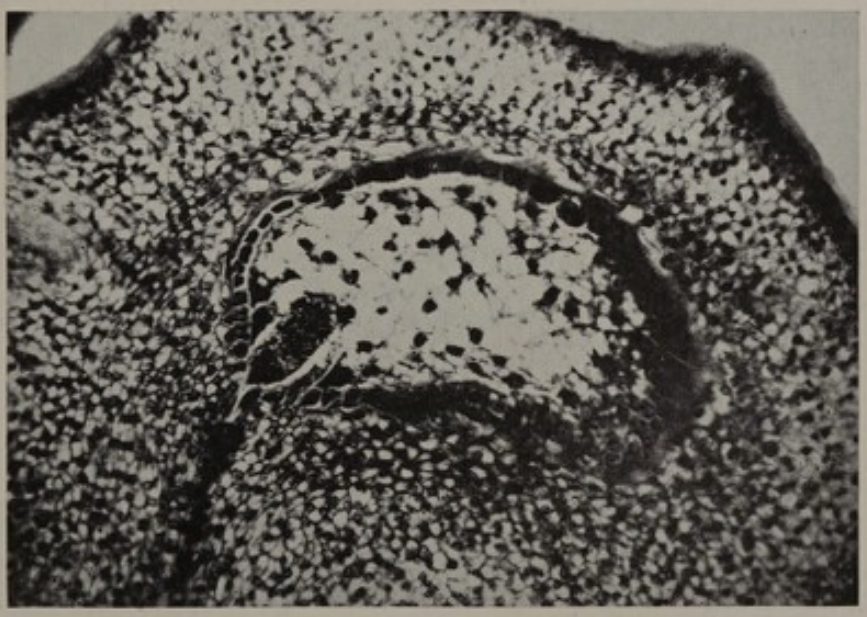

FIG. 15. Section of young ovule from an incompatible cross. Small embryo at left. Nurse cells at right enlarged and beginning to divide.

FIG. 16. Nurse cells in an incompatible cross increasing in activity. Embryo at top of embryo sac and its other contents are disintegrating.

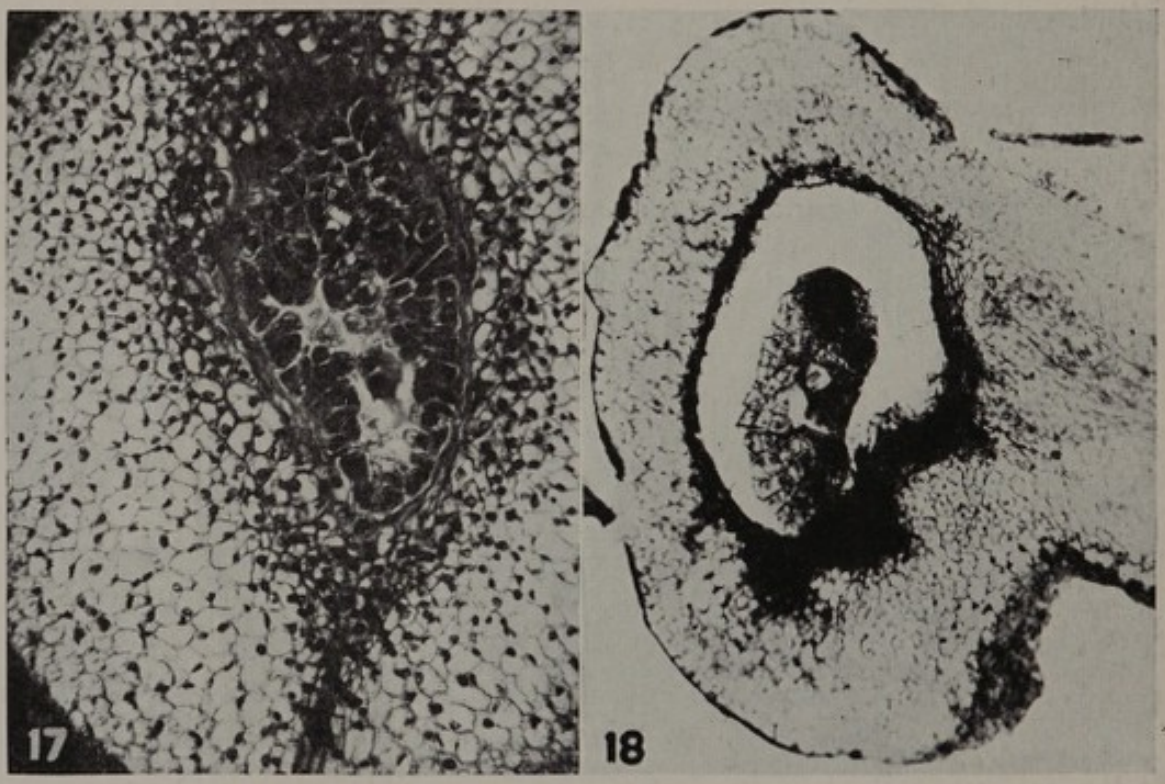

FIG. 17. Embryo sac completely filled by ovular tumor produced by nurse cells.
FIG. 18. Ovular tumor in the form of a "pseudoembyro."

equivocal at best for the presence of different enzymes acting on the different substrates.

Results with cyanide and cysteine indicated the absence of conventional adenylpyrophosphatase in the mitotic apparatus. Both these substances are known to inhibit alkaline phosphatase[17, 20, 23, 27, 28, 36] and to have no deleterious effect on muscle ATPase,[9, 42] chick embryo adenylpyrophosphatase,[39] or yeast apyrase.[29] With the mouse-tissue cultures, cyanide or cysteine at 10 and 50 mM inhibited the histochemical detection of phosphatase with ATP, muscle adenylic acid, and yeast adenylic acid as substrates.

Glycine, which Bodansky found to accelerate alkaline phosphatase optimally at 6.25 mM and to inhibit at higher concentrations, proved at 62.5 mM to inhibit the splitting of both yeast and muscle adenylic acids in the fixed mouse cells. The inhibition with the muscle adenylic acid was most evident when the substrate concentration was just above the threshold of detectability.

With respect to the greater ability of our cultured mouse cells to split muscle adenylic acid than yeast adenylic acid, it should be mentioned that Reis[33, 34] described a specific 5-nucleotidase in animal tissues capable of hydrolyzing adenosine-5-phosphoric acid and inosine-5-phosphoric acid, but not ATP. Such an enzyme has been suggested as effective in removal of the ester phosphate from the adenosine-5-phosphate formed from ATP by adenylpyrophosphatase in mouse epidermis and carcinoma[35] and in autolyzing dog brain.[18, 19] It would be interesting to speculate on the splitting of ATP in the fixed mouse fibroblasts. Is there first a removal of the two labile phosphate groups by alkaline phosphatase, followed by an ester hydrolysis with 5-nucleotidase? That much of the staining with ATP is perhaps attributable to hydrolysis of muscle adenylic acid present as a contaminant is suggested by the greater blackening obtained with the ATP preparations of suspected lower purity. The ATP could also be expected to hydrolyze spontaneously during incubation[16] to yield some muscle adenylic acid.

It would appear, then, that the presence on the mitotic chromosomes and achromatic figure of more than one phosphatase active in the alkaline range and on the substrates we have used is de-

batable. Besides alkaline phosphatase in these cultured mouse cells, there is better evidence for the presence of a 5-nucleotidase than for a specific adenosinetriphosphatase. The role of phosphatases in mitosis is not clear, but we may recall the suggestion that they are instrumental in nucleic acid metabolism,[21, 23, 30, 41] and, with respect to the spindle, that they may be engaged in a manner as yet not understood in the synthesis of fibrous proteins in general, such as keratin and collagen.[4, 10] If the differentially darkened regions of the chromosomes here interpreted as centromeres are really heterochromatin, as would appear likely from the appearance of resting nuclei, one is reminded of the suggestion by Goldschmidt and Lin that heterochromatin is a stuff necessary for mitosis.

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CHROMOSOMES, CHEMICAL STIMULATORS, AND INHIBITORS OF NORMAL AND ABNORMAL PLANT GROWTH

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It is not necessary, I believe, to offer an apology for speaking to this conference about problems of differentiation and growth in plants. There is more in common in the life processes of plants and animals than is often realized. It is not surprising, therefore, that principles discovered in one group of organisms have been found to be valid in other groups as well. As apparently the lone representative of Botany in this conference, I cannot resist the temptation to remind you of several fundamental principles in my own field of genetics that were discovered with plants. Mendel discovered the main mechanism of heredity with garden peas, although its importance was not realized in his time. It was rediscovered independently a generation later by three botanists. The Danish botanist, Johannsen, gave us the pure line theory—distinguishing what organisms appear to be (phenotypes) from what they actually are in hereditary constitution (genotypes). The botanist, de Vries, gave us the mutation theory from work with plants. He it was who also, in the last century, first foreshadowed our modern concept of the gene by arguing that all cells of an organism contain the same assortment of hereditary units. I recently ran across the printed address of de Vries given at the dedication of the Carnegie Department of Genetics in 1904. In this, he suggested the use of the rays of Roentgen and Curie to induce hereditary changes within the cells in what he called pangens but what we now call genes. This was nearly twenty-five years before Muller and some others of us began using irradiation to induce chromosomal and gene mutations without knowing of the suggestion of the botanist de Vries. It is not unlikely that some of these ideas that I have mentioned,

which came from a study of plants, will be found to influence the thinking and discussions in the various panels of this Conference on Cancer.

Dr. Little has suggested that I speak about chromosomal mutations—the effects of individual chromosomes and of major parts of chromosomes in distinction from mutations in the individual genes contained in the chromosomes. For study of chromosomal mutations we have found an especially favorable group of organisms in the genus *Datura,* the best-known species of which is the Jimson weed, *Datura stramonium.*

Figure 1 shows that there is one of each kind of chromosome in the egg and in the sperm cell formed by the pollen and that these $1n$ sex cells, by their union in fertilization, produce a $2n$ or diploid fusion cell that develops the $2n$ somatic cells of the adult plant. Each chromosome contains a different aggregate of genes, which is evident from the different types of fruits produced when one of the twelve chromosomes is present as an extra. Fruits of the twelve primary $2n + 1$ types are shown in Fig. 2, each of which has in its cells a single extra unmodified chromosome. The chromosomes are distinguished by models with numbered ends, the largest chromosome being numbered 1·2 and the smallest 23·24.

In Fig. 3 are shown, in addition to a fruit of the primary $2n + 1{\cdot}2$ type, fruits of its two complementary secondary types, $2n + 1{\cdot}1$ and $2n + 2{\cdot}2$, together with models and camera lucida drawings of their chromosomes. The extra chromosome of these secondary types is composed of a doubled half chromosome.

Figure 4 shows two tertiary $2n + 1$ types, in which the extra chromosome is composed of parts of two different chromosomes. In this case, the models represent the extra chromosomes as composed of parts of a white and a stippled chromosome. We had earlier found that when an unmodified 17·18 chromosome (the stippled chromosome of the diagram) is present as an extra, branches often crack on the upper surface and develop a mass of unregulated tissue. It was of interest to us, therefore, that the $2n + 1{\cdot}18$ type shown in Fig. 4 also developed such abnormal tissue, a fact that indicates that the effect was due to factors in the 18 half of the 17·18 chromosome.

Not only has the addition of extra chromosomes been found to bring about important changes in the structure of the plant involved, but also the subtraction of chromosomes from the normal complement disturbs the chromosomal balance and induces morphological effects. Figure 5 shows a green-stemmed branch on the left, which arose as a budsport from a plant that was hybrid for genes for purple and green stem. Study of the chromosomes showed that in the budsport, possibly owing to previous chloral hydrate treatment, the chromosome carrying the gene for purple stem was fragmented, and the fragment with the purple gene was eliminated. Budsports, which are common in certain plants, have been generally assumed to be due to gene mutations, but all those examined in *Datura* have been found to be due to changes in chromosome number. Many other examples could be given of the effects of addition and loss of individual chromsomes.

Figure 6 shows a potted plant of *Datura* with a normal $2n$ branch on the right and on the left a $4n$ branch in which the chromosome number has been doubled. As is usually the case, the early leaves of the $4n$ branches are roughened and contain a mixture of $2n$ and $4n$ cells. About ten years ago, we stumbled on the use of the alkaloid, colchicine, as an effective agent with which to induce chromosomal doubling in higher plants. Its use was suggested from its delaying action on nuclear divisions in animals, although it does not seem able to induce chromosome doubling in animal cells. Chromosome doubling is of interest to floriculture, since it usually increases the size of flowers as shown in Fig. 7, but its greatest value probably will come from its ability to change sterile "mule" plants into fertile, pure-breeding types that may represent new species.

As seen in Fig. 8, we have classified the chromosomal types in *Datura* into two main groups, the balanced types in which there is an equal number of chromosomes of each kind and the unbalanced types in which there is not the same number of chromosomes in each set. Table 1 is a summary made some years ago of the chromosomal types secured in *Datura* and of the types considered theoretically possible judging from those actually secured. It will

be seen that changes in chromosomal number are capable of producing a wide range of variation.

TABLE 1

SUMMARY OF CHROMOSOMAL TYPES, IDENTIFIED IN *DATURA STRAMONIUM* TOGETHER WITH NUMBER THEORETICALLY POSSIBLE

I = Primary (unmodified) chromosome. II = Secondary chromosome (with two like ends). III = Tertiary chromosome (with ends from two different primaries).

Chromosomal types	No. forms identified	No. forms theoretically possible	Formulae
1n, 2n, 3n, 4n	4	4	
1n + II	2	24	2n
2n + I	12	12	n
2n + II	14	24	2n
2n + III	25	264	2n(n-1)
2n + 2 I	2	12	n
2n + 2(I & II)	1	24	2n(I & II related)
2n + I + I	47	66	$\frac{n(n-1)}{2}$
2n + I + II	9	264	2n(n-1) (I & II unrel.)
2n + I + III	2	3168	$2n^2(n-1)$
2n + I + I + I	1	220	$\frac{n(n-1)(n-2)}{6}$
2n − I	2	12	n
2n − I + II	1	24	2n(I & II related)
2n − I + II + III	7	264	2n(n-1)
2n − I + III + III	21	264	2n(n-1)
2n − I_2 + III_2 + III_2	1	264	2n(n-1)
2n − I + III + III + I	2	3168	$2n^2(n-1)$
2n + 1 free fragment	9	24	2n
2n + 2 free fragments	4	24	2n
2n − I + II + free fragment	1	24	2n
2n − I_2 + II_2 + (free fragment)$_2$	1	24	2n
2n − I + III + free fragment	11	24	2n
2n − I_2 + III_2 + (free fragment)$_2$	3	24	2n
2n − I − I + III + III + free fragment	2	528	4n(n-1)
2n − I_2 − I_2 + III_2 + III_2 + (free fragment)$_2$	1	528	4n(n-1)
2n + 1 translocated fragment	1	24	2n
2n + 2 translocated fragments	1	24	2n
2n − I + II + translocated fragment	1	24	2n
2n − I + III + translocated fragment	2	24	2n
2n − I_2 + III_2 + (translocated fragment)$_2$	1	24	2n
3n + I	1	12	n
3n − I	1	12	n
4n + I	8	12	n
4n + 2 I	1	12	n

TABLE 1—*Continued*

Chromosomal types	No. forms identified	No. forms theoretically possible	Formulae
4n + 3 I	1	12	n
4n — I	5	12	n
4n + I + I	1	66	$\frac{n(n-1)}{2}$
4n + I — I	1	132	n(n-1)
4n — I — I	1	66	$\frac{n(n-1)}{2}$
4n + I + I — I — I	1	2970	$\frac{n(n-1)(n-2)(n-3)}{4}$

Under forms theoretically possible only morphological types are included. If arrangement of chromosomal parts were considered, some figures would be greatly increased. All calculations assume that the two parts into which a chromosome breaks are always equal. Hence the figures in the table form a minimum.

We have been discussing conditions in our plants in which it is fairly clear that the differences in differentiation and growth are in some way controlled by the chromosomes. I wish now to say something about a condition in which the controlling influence is perhaps tied up in some way with nonchromosomal constituents of the cell and with the internal microenvironment.

In Fig. 9 are shown fruits of the ten herbaceous species of *Datura* which we have in cultivation at the Smith College Genetics Experiment Station. In order to study the chromosome differences between these species, it was desirable to secure hybrids between them and *D. stramonium,* which was used as a standard. Unfortunately, most of them would not hybridize. Growth of hybrid embryos has been found to be an important barrier to crossability between species. Figure 10 shows outlines of various stages in growth of normal embryos of *D. stramonium* from the 7-day embryo of about 0.1 mm. in diameter through the heart and torpedo stages in the upper row to the adult embryo with curved cotyledons at the lower right. In co-operation with Dr. van Overbeek and Dr. Conklin, techniques were developed for culture of such normal embryos on artificial media. It was found that coconut milk as a stimulator enabled us to cultivate much younger embryos, as small as 0.1 mm. in diameter. Later, we found that malt extract when

sterilized through a Seitz filter was equally effective as a stimulator of embryo growth.

Figure 11 on lower left shows fruits of *D. ferox,* and on the right, those induced by pollinating this species with pollen of *D. meteloides.* No seeds were produced from this attempted cross, and no embryos were found on dissecting the ovaries. On the upper row, at the right, is shown the result of a cross between *D. stramonium* and *D. metel.* A few seedlike bodies were formed but never has this species combination yielded a seed capable of germination. Sometimes, however, before the seedlike body aborts, a hybrid embryo can be secured by a cesarean operation and brought into cultivation on artificial media, and induced to continue development like an incubator baby. Some such hybrid embryos from different crosses are shown in Fig. 12. By their artificial cultivation, it has been possible to induce them to develop into mature plants which often show considerable hybrid vigor. As seen in Fig. 13, it has become possible by the artificial culture of hybrid embryos to secure many new hybrids, which were otherwise impossible.

The problem arose as to what might be the factors that prevent the development of so many hybrid embryos after fertilization has occurred. In some combinations, only a few cell divisions take place after fertilization, whereas in other combinations, the embryos develop to the heart or even the torpedo stage before their growth becomes arrested. Apparently embryo abortion is connected in some way with abnormal growth of one of the tissue elements of the young ovules. In Fig. 14 is shown a section of a young normal ovule. Unfortunately, the heart-stage embryo had dropped out from the section but the place is indicated by the hole near the center. Of particular interest for our discussion is the single layer of cells that surrounds the embryo sac and separates the large cells of the endosperm inside the embryo sac from the smaller cells outside. Dr. Satin has found that this layer, technically known as endothelium but which we can call nurse cells from their apparent function, regularly occurs in normal selfed seeds of different species of *Datura.* The cells outside this layer of nurse cells are at first gorged with starch grains. As the embryo develops, the starch granules of these outlying cells are used up, and through the

action of the nurse cells, this food is passed on to the developing embryo. In incompatible crosses, however, the behavior is quite different. The starch in the storage cells is not digested and the nurse cells, which normally remain a single layer only, begin to divide in all directions and may form a proliferating mass, which we have called an ovular tumor. Figure 15 shows a small embryo at the left and the layer of nurse cells beginning to divide at the right. In Fig. 16, the dividing nurse cells are becoming active and the embryo at the top of the embryo sac and its other contents are disintegrating. In Fig. 17, the embryo sac is completely filled by the ovular tumor. Figure 18 is a later stage in which the ovular tumor has taken the form that we have called a pseudoembryo.

Dr. Rappaport has found that the jelly-like mass containing embryo tumors and adjacent tissue that is produced in an incompatible cross can be extracted to give a water-soluble thermostable inhibitor. This inhibitor prevents the growth of normal embryos of *D. stramonium* when they are cultivated in artificial media. It also arrests growth of normal embryos when it is injected into young ovaries. Preliminary tests seem to indicate that tissues associated with ovules the development of which has been arrested by the extracts in vivo also produce an inhibitor that can be extracted and used to inhibit embryo growth in other ovaries. If these findings are confirmed by further tests, we may have evidence that we are dealing in the embryo inhibitor with a self-multiplying substance. We have spoken of this water-soluble extract as an inhibitor. It may also be considered a stimulator. There is evidence that the same substance may act differentially and stimulate one tissue (in this case, the nurse cells) and at the same time directly or indirectly inhibit another tissue (in this case, the cells of the hybrid embryo).

Photospectroscopic tests seem to indicate that the embryo inhibitor is a nucleoprotein. Attempts are being made to learn more about the nature of the inhibitor and its effects. A number of nucleoproteins are being tested for possible similar action. Efforts are being made to discover some substance or condition that can destroy, neutralize, or inhibit the inhibitor. Toward this end, we hope to be able to grow the nurse cells in tissue culture and try

different agents on them in an effort to control their growth in vitro and then possibly in vivo later. I judge from conversation on the train with cancer investigators that tissue cultures of animal origin tend to retain their specificity.

CONCLUSION

If we succeed in our efforts, we may be able, on the plant side, to remove an important barrier to crossability and thus greatly increase the number of wide species hybrids that can be secured. We trust that, in so doing, we may also turn up some facts of interest to students of abnormal growth in animals. Aside from these possible practical applications, we are more especially interested in gaining an insight into how growth and differentiation are initiated and controlled in living systems. This we believe is the outstanding problem in biology. We shall be grateful if our work with plants may make a modest contribution to the solution of this major problem.

III

Nature of Vertebrate Individuality

THE PROBLEM OF CELLULAR DIFFERENTIATION*

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What is needed in the study of growth and differentiation is not only the accumulation of more data, but a more precise description and more penetrating analysis of the data in a more rigorous conceptual frame of reference. This need calls for a firmer trend away from obscurant verbalisms toward objective scientific formulations of the problems involved. We cannot hope to develop a better understanding of the phenomena of growth, differentiation, organization, induction, control, harmony, and so on, unless we first obtain a realistic picture of just what factual content these various labels cover. By way of example, such an operational analysis is attempted here for the problem of cellular differentiation. It is a condensed and combined version of results reported and thoughts expressed in three previous publications.

WHAT IS DIFFERENTIATION?

The term "growth" may be reserved to designate the increase in protoplasmic mass, that is, the production, or rather reproduction, of more units of a given kind. This may or may not be associated with a further subdivision (by cell divisions) of the growing mass. As long as the resulting fractions retain essentially the same constitution and appearance, we may speak of pure growth. "Differ-

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entiation," on the other hand, connotes the appearance of systematic differences among parts that were originally of the same kind. If "growth" means more of a kind, "differentiation" means more kinds. In cellular terms, true "differentiation" then implies the real, not only apparent, diversification (i.e., divergence of character) of cells or cell strains that initially were alike, rather than just looked alike.

DOES TRUE DIFFERENTIATION EXIST?

Whether differentiation in this strict sense really exists, is an empirical question. The answer hinges on the reliability of our testing methods, which furnish the criteria by which to tell the likeness or unlikeness of cells. Historically, undue faith in microscopic criteria has confused the issue. Cells that looked alike were rated as similar, and cells that looked different were considered to be intrinsically different. We must rid ourselves of this ingrained, but utterly fallacious, habit of judging by appearances. A cell is a going concern, in constant interplay with its environment. Its microscopic equipment is merely the cumulative record of its reactions to this environment, reactions of a specifically constituted system to the physicochemical conditions prevailing in the surrounding space. All optically or otherwise discernible characters of a cell are the results and residues of anteceding formative processes. They are indexes, at best, of certain cellular activities only; namely, those that do express themselves morphologically. Since many cellular reactions leave no morphological trace, differentiation of cell character cannot be defined in terms of microscopic criteria. A complete characterization of a given cell would have to include a complete inventory of the molecular species present, their combination and distribution in space, and a list of all possible reactions and manifestations of which the system is capable under any conceivable conditions (the "response repertory"). This seems unattainable in our present state of knowledge. Yet, we can at least exploit to a greater extent than heretofore those tests of cell behavior that are practicable. If we then compare the behavior of two cells or cell strains under identical conditions, e.g., in a common medium, and note constant differences in their behavior

in one or more regards, we must conclude that they are of different character, even if they appear alike under the microscope. Conversely, if two strains that differed markedly in appearance while in the organism behave identically when brought into the same medium, this proves conclusively that they have been of one type and had merely displayed different portions of their response repertory in the face of different local environments. The latter process, exemplified by the transformation of fixed histiocytes into macrophages, has been termed "modulation," in contradistinction to "true differentiation" which implies an irreversible change in constitution.

On the basis of such behavioral tests, the occurrence of true differentiation during the development of all higher animals must be affirmed. Tissue culture has shown that cell generations derived from different organs, in spite of assuming similar appearances, retain many of the specific properties of the original strains. This means not only that they had differed in their protoplasmic constitution at the time of explantation, but were able to persist in synthesizing the same specifically different protoplasms without reverting to common type. On the other hand, since the evidence of Experimental Embryology proves that such different cell strains have originated from a common stock, the intrinsic diversification of cell strains during development is an incontrovertible fact. Since this diversification occurs by degrees, it is incorrect to speak of any cell as being "undifferentiated" or "embryonic." Differentiation is not an all-or-none reaction, but a long chain of progressive transformations, so that any cell we are considering has reached a certain point along that line. Also, at each point of the line, the cell is capable of a variety of reactions only part of which are compatible with harmonious development. At any step, an abnormal contingency may provoke a response that may throw the further course of the cell strain off balance and lead to pathological effects.

THE RESPONSE REPERTORY

If, according to the foregoing, visible criteria of shape, arrangement, and so on, are unreliable and incomplete tests of differentiation, what other means of detection do we have available? There

are first the chemical products of cells, such as fibers and secretions. Different products are often valid indicators of intrinsic differences in the production plant. Histological stains also sometimes provide sensitive microchemical tests of cellular differences. Histochemical techniques, although still crude, likewise demonstrate specific differences among cells. Further constitutional differences may be revealed by the differential reactions of cells to drugs, hormones, or radiations, provided possible errors due to unequal exposure can be excluded. In this manner, much subtler differences can be detected than are morphologically indicated; for instance, constitutional differences among cells on different branches of the vascular tree, among different types of nerve cells, among different areas of an epithelium, or different regions of the connective tissue.

If this constitutional divergence among cells is based on, or at least associated with, the appearance of distinctive cell proteins, it might be possible one day to trace it by immunological methods. Antiserums against extracts of sperm, lens, kidney, or reticuloendothelial tissue have been shown to have a selective action on homologous organs. We have seen evidence of similar effects in the embryo. We are now testing the possibility of selective absorption of organ antiserums (tagged by C^{14}) by the embryonic precursors of the homologous organs as a means of tracing back the first stages of biochemical divergence. Still other methods are on the horizon. Combined, such methods will give us more pertinent information on when the crucial steps in the differentiation of a cell strain take place. Only then will our attention become more properly focussed on the process of differentiation rather than on the products of differentiation with which we are mostly preoccupied at present.

MOLECULAR ECOLOGY OF THE CELL

The study of cell behavior in development, in immunological reactions, and in the response to drugs, has drawn increasing attention to the cell surface as the seat of specificity of interaction between cell and environment. Similarly, interfaces in the interior appear as seats of specific interactions in the intracellular, intra-

nuclear, etc., spaces. It seems that mere colloid-physical considerations do not provide reaction mechanisms of sufficiently subtle specificity to account for the highly specific and selective interactions recorded in these fields. Interest, therefore, has turned toward intermolecular forces producing bonds of varying strength depending on the configurational fitting ("steric conformance" of a key-lock type) between the respective molecules (e.g., Pauling). Cell relations would be controlled by the interlocking of complementary compounds. The response repertory of a cell would be limited by the number of such key species present in the cell. Not all of these, however, will become effective. In order to operate or combine, conditions for their operation or combination must be favorable. Interfaces offer such favorable conditions by adsorbing, concentrating, and orienting molecular films. The main point is that in this they act selectively. That is to say, depending on the physical and chemical conditions along the interface, certain segments of the molecular population will be selectively attracted to the exclusion of others. Two cells, otherwise identical, confronted with differently constituted interfaces, therefore develop surfaces of qualitatively different composition, and as a result diverge in their subsequent reactions. It should be clear, even from this very condensed comment, that what really counts in determining cell fate is not just the type of chemical compounds present but which of them are in operative condition. And it is in this respect that the physical constellation in the system becomes of paramount importance in setting the stage for the biochemical events. Disposition in space becomes as significant as chemical composition. The field of study investigating this complex but orderly behavior of molecular populations in cells may be termed "Molecular Ecology."

WHERE DOES DIFFERENTIATION OCCUR?

In the light of the concept just outlined, the first step in the differentiation of a cell may be envisaged as consisting of the selective concentration in its surface of certain specific key compounds, which then by virtue of their unique position and orientation act

as anchor points for further molecular apposition and also perhaps as catalysts of specific reactions. A period of lability gives way to gradual consolidation, marking the appearance of irreversible features. The evidence of genetics indicates that the genic equipment of the cells of all tissues is and remains the same throughout development, hence, is not subject to differentiation. Differentiation seems confined to the extragenic protoplasm. At the same time, the number and type of differentiations a cell can undergo is strictly limited by the hereditary endowment of the species. Yet, once a cell has attained a given state of differentiation, it can pass this on without attenuation to generations of descendent cells, as is evidenced by tissue-culture experiments. These seemingly conflicting statements can be readily reconciled on the basis of the preceding remarks, leading to the following concept. 1. The numbers and kinds of key compounds that can be synthesized by a given cell are determined by the genic endowment. This assortment is the material basis of what we used to call cell "potency." 2. In any given case, only a fraction of this is "activated," that is, given opportunity to become effective, by being adsorbed to a surface or otherwise enhanced. It becomes the molecular master population of that cell. 3. This master population would then impose its pattern on the further course of synthesis of protoplasm.

A cytoplasmic master compound could perpetuate its kind in one of two ways, depending upon whether the genic apparatus generates the full assortment of terminal products or merely gives rise to more primordial compounds from which the terminal molecular specialties have to be derived by secondary degradations and conversions. In the former case, the cytoplasmic master compound would build on ready-made units of similar kind, while in the latter case, it would impose its own pattern upon the primordial compounds, template fashion. Thus, although at their source in the nucleus, all basic protoplasmic units may be identical in all cells, they would, on contact with the differentiated populations of the cytoplasm, assume the special characters of the latter. The stuff, in this view, is furnished by the nucleus, but reshaped by the differentiated cytoplasm acting as model. The perpetuation of cyto-

plasmic specificity is thereby insured as long as the nuclear production site remains trapped inside the cytoplasm. Suggestive support for this view is found in the work of Caspersson on the nuclear production site of proteins, as well as in our own demonstration that the synthesis of protoplasm in nerve fibers occurs exclusively in the nucleated central portion of the neuron. The demonstration by Sonneborn of self-perpetuating, although gene-dependent, cytoplasmic bodies in *Paramaecium* seems capable of a similar interpretation and may, as he suggests, have a bearing on the mechanism of cellular differentiation in higher forms.

In conclusion, differentiation is a process in which different parts of the cell system play different roles, and further research is needed to identify and clarify the component processes involved.

CONTACT HARMONY

Among the least patent criteria of differentiation is the sum total of properties that permit a cell to live in harmony with its neighbors of the same or other types. This is due not merely to the rapid elimination of all disharmonious combinations if and when they occur, but to the subtle preadaptation of cells to one another's prerequisites. One of the most striking examples is the ability of cells to form tissues by (1) aggregating with their own kind, (2) combining with complementary types (e.g., epithelium and mesenchym; nerve fibers and sheath cells), and (3) rejecting association with foreign types. That such associations and separations are not simply the accidental result of proliferation of continuous masses from common centers, but involve active selectivity, is clearly brought out by the selective fusion of identical and complementary tissues in regeneration, wound healing, transplantation, and in the reorganization of cell groups after forcible dissociation. New investigations on this problem are being carried on in my laboratory. Evidently, cells possess a high degree of discriminatory ability in recognizing each other. Their means of recognition must be situated on the surfaces, for this is where they make contact. Contact may occur between naked protoplasts or through an intervening coat of exudate. As suggested earlier, the "means of recognition" may consist of the specific shapes of molecules ex-

posed to the surface. Specific links between conforming molecules could then establish a cohesive union between adjacent cells. Secondarily, such groupings may be consolidated by the formation of fibrous skeletons and membranes. While calcium ions seem to play an important part in promoting adhesiveness between cells, they can evidently not be the determining factor in the selectivity of the associations.

Contact affinities and disaffinities between cells develop in the course of development as corollaries of differentiation (Holtfreter). Thus, two cell strains from different sources can independently develop complementary characters that predispose them for a later union. In other instances, cell types may become mutually adapted after coming in contact. In still other cases, one more advanced cell type may force a less consolidated neighbor into a conforming state, as occurs in the "inductions by contact" to be discussed presently. The final outcome is always harmony of interactions between contiguous cells, as well as between cells and their medium. Establishment of such harmony terminates cell locomotion. Disturbance of the harmonious state sets the cell on the move again. This explains the restlessness at the free edge of an epithelial sheet and similar phenomena. Cells keep moving until all their specific surface contacts are properly matched. As contact harmony depends upon a great many factors, so disharmony may result from a variety of causes. It may originate in a change in the cellular environment or in a change in the cell itself. If the latter change is of such a kind as to reduce the discriminatory acuity that rules in the association among normal cells, inconsistent and abnormal groupings may ensue.

The bearing of these matters on problems of cancer, particularly invasiveness and metastasizing faculty, is evident. To the extent to which these properties mark misdirections of the differentiation process, cancer is a problem of differentiation rather than of growth. The concept outlined in the preceding section explains in principle how a cytoplasmic deviation, once it has occurred, can be perpetuated throughout the subsequent growth of descendent cell generations.

CONTACT INDUCTIONS

The work of Spemann and others has shown that a more mature tissue may influence the course of development of a less differentiated tissue with which it comes in contact. The nature and specificity of this action has long been in the center of interest. That chemical interaction is somehow involved has long since become clear. The underlying mechanism, however, is still as obscure as ever. The immediate effect does not seem to be of the diffusible kind, for it requires that the affected cells be in intimate contact with the inducing cells or, at least, with some cell debris. In the study of one such pair of dependencies, namely the induction of the lens by the eye cup in the chick embryo, we have noted that the first visible trace of the effect consists of a sudden orientation of the prospective lens cells relative to the inducing retina, with the axes of the former becoming perpendicular to the contact surface. Since this reorientation coincides with the area of contact, it must be interpreted as a transcellular contact effect. It is presumably but a sign of the reshuffling and segregation of the molecular population in the epidermal cells in the sense already indicated, that is, attraction of key molecules to the new contact area followed by oriented adsorption, the building on of oriented chains of molecules, and consequent redisposition of the chemical systems of the cell. It is noteworthy that we have seen a similar reorientation of epidermal cells in larval amphibians within a few days after local exposure to an implanted crystal of the carcinogen, methylcholanthrene. Many other instances of contact induction are equally suggestive. Whether or not the particular explanation, attempted here, will prove to be tenable, it shows at least the type of approach from which an eventual resolution of such terms as "induction" into physicochemical realities may be expected. Many pathological processes have long been known to be associated with peculiar cell arrangements, e.g., "pallisading," but little has been done to exploit the descriptive-morphological facts as clues to an understanding of the underlying molecular processes.

A further experimental analysis of the relation between differentiation and contact action seems feasible by exposing cells to

surfaces of defined constitution. Nageotte has demonstrated the ability of alcohol-fixed cartilage to induce additional cartilage formation in the surrounding tissue. I have described the specific induction of new cartilage along the surface of implanted cartilage that had been devitalized by quick-freezing and drying, as well as induction of cornea and nerve sheath after similar treatment of the corresponding tissues. The specificity of tissue inductions following injection of tissue extracts, as described by Levander and others, remains to be confirmed. Yet, more intensive work along these lines holds promise of valuable data of general significance for the problem of differentiation.

In all such cases, one will have to distinguish clearly between truly differentiating effects, which push a cell into one of several alternate courses, and mere realizing effects, which permit an already single-tracked cell to express some previously latent character. Early embryos mostly furnish examples of the former category, older animals of the latter. A good case in point are the hormones, which in most instances act by merely promoting or repressing in the target cell a course of differentiation the character of which, including hormone-dependence, has been determined by prehormonal influences.

CONCLUSION

This brief survey may suffice to give an idea of the highly composite nature of the phenomena described as "differentiation" and the multiplicity of factors involved. If a cell deviates in its course of behavior from our expectations, we call it pathological. But we realize that the number of points at which such deviations may occur is as great as the number of steps in the process of differentiation. At each step, the deviation may be due to an aberrant change within the cell system or outside of it. Within, it may originate in the cytoplasm, in the nucleus, or in the genes; outside, it may arise in the immediate contact environment (another cell or surrounding matrix) or may come from a distance (as in the case of diffusible agents). It may imply a trivial deficiency easy to repair, or a profound constitutional alteration doomed to permanency. It may affect chemical composition or simply the realization of

physical conditions necessary for given chemical systems to become operative. It may act at the surface or in the interior, affect equilibriums of concentration or conformance of configuration, production of compounds or their distribution, electrical or mechanical properties, mobility or adhesiveness, and so on. Realizing this diversity of factors, nothing could be farther off the mark than trying to embrace differentiation—or for that matter, its pathological variations—in a single formula, as do those who search for, or speculate about, "the" mechanism of differentiation, as well as those who speak of "differentiated" and "undifferentiated" cells, as if these were just two sharply delimited stages. This habit of dealing with "differentiation" as a rather vague generalization has seriously handicapped the breaking down of the general problem into concrete and tractable parts which would lend themselves to experimental attack. The purpose of this paper has been to present illustrations of the feasibility and advantages of a less abstract and more factual approach.

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GENETIC EVIDENCE OF VERTEBRATE INDIVIDUALITY

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A belief in the principle of individuality within the vertebrates, as in nearly all the higher plants and animals, would seem to be obvious to a student of genetics. Assuming that the number of genes in any of these species will be well over a thousand, probably between five to ten thousand, the number of possible combinations of alleles with contrasting effects is so great that only rarely within a species will the same combinations of genes be duplicated in any individuals. Identical twins in animals and plant varieties that are self-fertilized represent examples of exceptions to the general rule of individuality that would be expected from different combinations of genes.

If one accepts the premise of more or less universal individuality, the question naturally arises as to how it can be measured. In humans, members of a particular race practically never have difficulty in distinguishing between individuals of their own race on the basis of external appearance but may make many mistakes in differentiating individuals belonging to another race. Certainly, some more objective index of individuality is required than similarities or dissimilarities of morphology.

TISSUE TRANSPLANTATION

Probably the earliest method that has been used for the study of individuality in various species of animals is that of transplantation of tissue between species or between individuals. The vast amount of experimental work in tissue transplantation bearing on this subject has recently been summarized by Loeb. A general conclusion, which seems justifiable from all these experiments, is that the closer the relationship between the donor of the tissue and the host, the greater is the chance of a successful take of the trans-

planted tissue. Many years ago, Little proposed an explanation of a genetic character that required for its manifestation the simultaneous presence of more than one mendelizing factor. This proposal seems particularly applicable for the explanation of the successful take of transplanted tissue, whether cancerous or normal. That is, if the transplant contains one or more gene products not present in the host tissue, the graft presumably will not be successful.

ANTIGENIC SUBSTANCES

Another definite measure of individuality within a species has been obtained by use of the techniques of immunology on the red blood cells. The discovery by Landsteiner of the so-called blood groups of the red blood cells of humans has represented a milestone in both immunology and genetics—in the latter, because of its widespread use in the study of heredity in man. As is well known, every member of the human race belongs to one of the four groups, A, B, AB, or O. The later discoveries of the subgroups A_1 and A_2, of the contrasting pair of substances M and N, of P, and more recently of the Rh-Hr antigens, have increased the number of combinations of antigenic characters very greatly, and thereby made more plausible the concept of complete individuality within humans. The role of the Rh-Hr substances in hemolytic disease of the newborn is so well-known that further reference to it is needless.

In our laboratory, early attention was given to a genetic analysis of differences between species, utilizing immunological techniques, and using for experimental material the species hybrids and backcross hybrids in pigeons and doves produced by the late Professor L. J. Cole. Briefly, it was found that the hybrids between two species possessed in their red blood cells all or nearly all the substances of both parental species. Furthermore, in one species cross between an Asiatic species, the Pearlneck *(Streptopelia chinensis),* and the domesticated Ring dove *(St. risoria),* the hybrids did not contain quite all the specific substances of either parental species and did possess a complex of antigenic substances not found in the cells of the parents. This has been called the

"hybrid substance," and has been present in every hybrid produced between these two species. Presumably, it is caused by the complementary action of genes from each parent, which, by their interaction in the species hybrid, produce a different substance than either can produce alone. Not all hybrids between various other species possess a hybrid substance, making it appear reasonable to conclude that only particular complexes of genes may interact to produce a new antigenic substance.

Upon repeatedly backcrossing these species hybrids and selected offspring to the Ring doves, ten antigenic substances that differentiated Pearlneck from Ring dove were eventually isolated as probable units. That is, a backcross bird carrying any one of these so-called unit antigens, when mated to a Ring dove, produced equal proportions of progeny with, and without, the particular character in their blood cells. One may conclude then, that one or more genes on each of ten chromosomes produce effects that distinguish Pearlneck from Ring dove. The antigenic substances peculiar to Ring dove, in contrast to Pearlneck, have not been identified as units. However, the available evidence indicates that a gene or genes on nine or ten chromosomes of Ring dove produce effects that differentiate that species from Pearlneck. The antigenic substances that are shared by the two species have not been identified as units, and no information exists as to their number. It seems probable, however, that they are fully as numerous, if not more so, than those that set each species apart from the other. Presumably, the common antigenic substances are gene-determined, although whether the causative genes are linked to or independent of those producing the species specific substances is unknown. Parallel findings have been made on other species crosses, thus strengthening the proposal that there are genes on many chromosomes of a species with antigenic effects on the blood cells; quite possibly, therefore, on other tissues as well. If the effects on the cells of a sufficient number of genes within a species could be recognized, an objective test of individuality would be available. (References to the papers dealing with the experimental evidence for these statements are given in a recent review.[3])

The findings in our laboratory have revealed that complete or

almost complete individuality of the red blood cells of cattle is a reality, to mention only the species most thoroughly studied to date. At present, forty-odd antigenic substances in cattle cells are recognized by virtue of the reaction of each of these with a specific reagent, or test fluid. (These reagents are prepared from isoimmune serums, or from antiserums from rabbits.) They have been labeled A, B, C, . . . Z, A′, B′ . . . L′ and Z′, respectively; subtypes have also been noted for certain of these. Each of these antigenic characters, when considered by itself, behaves in inheritance as if produced by a single gene and has appeared in an individual only if one or both parents possessed it. If each of these forty-odd substances were inherited independently of the others, the number of combinations possible would be almost unlimited.

However, it was first noticed by Stormont[14] that certain of these antigenic components appeared only if one or more other components were also present. For example, the antigen called "B" occurs alone, as does that called "G." But another cellular substance called "K" has never been observed unless both B and G were also present. (The only possible exception to their simultaneous appearance was noted shortly after the discovery of these antigenic substances, and was most probably due to a weak reaction with a B reagent, which was incorrectly recorded.) This association of K with B and G has occurred in more than a thousand animals of more than six thousand tested. Hence the combination of the BGK substances has always behaved as a unit, as have many other combinations of antigenic substances. It should be kept in mind that each constituent of a combination is recognized by virtue of its reaction with a specific reagent. These combinations of antigenic substances are therefore called "antigenic complexes."

There are two series of such complexes. In one series, there are twenty-one of the forty-odd antigenic characters that are associated in various combinations. Seven of these may appear singly, as was described for B and G. The other fourteen have been found only in various antigenic complexes, each of which may be made up of from two to eight of the twenty-one characters. The majority of these twenty-one characters do not occur at random in a complex with each of the others. The character K, for example, has always

been found with B and G, but never with the substance I. But either B or G may be present in a complex with I. Since no separation of the antigenic characters of a complex has ever been observed in the cells of the offspring of an individual possessing it, it seems somewhat more reasonable to assume that each antigenic complex is produced by a single gene than by linked genes. The various antigenic complexes in each of the two systems, or series, would then be produced by a series of multiple alleles.

A knowledge of the relationship of the so-called component parts of the complexes should provide information concerning the action of the causative genes. For example, are the substances B and G the same, or only related, when they occur alone and when they are in a complex, as in BGK? If they are the same substances in the complex as when they appear alone, it seems that the allele that effects BGK can produce not only the same substances as the respective genes for B and G, but the substance K in addition. Under this explanation, there would be many genes in this allelic series that could do the work of two or more alleles which themselves produce contrasting substances. Very much the same relation of the genes involved would obtain if the substances in a complex were not identical with, but closely related to, those that appear singly. Another possibility is that each antigenic complex has multiple or potentially multiple serological properties with sufficient similarities in antigenic structure to other complexes to make for overlapping specificities. Tests are under way to determine which of these explanations is the most reasonable.

Owing to the fact that more than half of these forty-odd antigenic characters are found in one or the other of the two series of complexes, the number of possible combinations is much less than would obtain if each were independent of the others. Nevertheless, except among closely related individuals, almost complete individuality has been noted of the cells of several thousand animals tested to date. If further substances are detected, as seems probable, the concept of complete individuality within this species may well be realized.

At the outset of these experiments, it was tacitly assumed that

in cattle, as in man, identical blood types would be found in identical twins, and that the blood types of nonidentical twins would be no more alike than those of siblings. In fact, the writer advised a colleague in another institution that a pair of twins was probably identical because of identity of blood types, and was promptly informed that this set of twins was far from identical in appearance. Somewhat later, this naïve assumption was upset still further by the results of tests on twin calves from a case of superfecundation, in which a genetic differentiation of the twins was possible. These twins, although differing in an external genetic character, had identical blood types, and the cells of each twin possessed two antigens, the genes for which could not have come from its own sire or from the dam.[12] Further tests on more than a hundred twin pairs have shown that about 90 per cent have identical blood types, whereas only about 6 per cent of twins are estimated to be identical. What appears to be the only reasonable explanation of identity of blood types in the majority of twins in cattle is that the embryonic union of the circulatory systems[7] provides a mechanism for the reciprocal migration of the so-called primordial blood cells and their establishment in the hematopoietic tissues of the co-twin. Thus, in such twins, there is a mosaic of blood cells—those that are formed by the hematopoietic tissues of the animal itself and those that are formed by the tissues derived from the co-twin.

One exception has been noted to the general rule that the majority of twins in cattle have identical blood types. A character called J is identified by its reactions with a normal antibody of cattle serum and behaves as if produced by a single gene. The cells of a pair of twins with a definite admixture of blood were tested, and all the cells of one twin carried the J substance, while none of the cells of the co-twin possessed it. Since the J character is poorly expressed, if at all, on the cells of a young calf, it appeared that this substance must have been supplied to the cells from some source other than the hematopoietic tissues.[13] It was found to be present in the plasma. When large quantities of blood carrying appropriate antigenic substances as markers, but not the J substance, were transfused into animals possessing J in both cells and

plasma, the transfused cells gradually "acquired" the J character. Furthermore, under favorable temperatures, non-J cells gradually took up the J substance in vitro when in contact with plasma containing J. Thus it seems that the non-J cells either affixed to themselves the substances from the plasma, or the plasma containing J effected some change in the non-J cells so that the J antigen became reactive with its specific antibody. The gene producing the J substance seemingly is not active in the hematopoietic tissues but produces its product elsewhere. Tests are under way to determine the place of origin of this substance, its chemical nature, and so on.

As a result of the studies in different laboratories on the antigens of the blood cells, it is reasonably certain that they contain numerous substances that are gene-determined. It requires no stretch of the imagination to assume that the cells of various tissues of the body will have either the same, or other, substances, likewise produced by genes. As more of these are detected, they may well aid in studies of the puzzling problem of the differentiation of the organs of the animal body with their respective specificities. It seems that they are adequate to explain the success or failure of tissue transplantation, except as that may depend upon a difference in antigenic substances between the tumor tissue and that of the individual in which it arose. The work of Gorer[1, 2] and Lumsden[10, 11] respectively have correlated one or more antigens of the blood cells in different species with the success of a transplanted tumor. That of Kidd shows how the techniques of immunology may aid in the study of the Brown-Pearce tumor in rabbits.

CONCLUSION

In conclusion, I should like to draw your attention to a possible parallel in the cancer field and in the study of cellular antigens in cattle twins. Within my knowledge, it would be generally accepted that it is possible to immunize members of an inbred strain of animals against a tumor that arose in an individual of that strain, at least following several or more generations of transplantation of the tumor. There is no evidence, however, that an animal has

an immune response to its own spontaneously arising tumor. Likewise, in cattle or other animals, one would anticipate some immunological response of an animal upon being transfused with cells carrying substances foreign to it. But in fraternal twins with an admixture of blood, there is at present no reason to believe that there has been an immune response to these so-called foreign cells from the co-twin. The question may be raised whether the biological basis for the presumed lack of immune response in these two cases is the same.

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IV

Functional Endocrinology

ANDROGENS AND GROWTH PHENOMENA

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Growth in the higher organisms is to a large extent a function of the endocrine system. Although the internal secretion of the pituitary gland appears to provide the basic stimulation for normal somatic growth—and for the "giant" type of abnormal growth when secretion of the growth hormone is overabundant during early life—other glands of internal secretion contribute hormones of essential value to growth; the pituitary gland exerts its growth-promoting effects when the basic substrate is in a physiological condition capable of response. The thyroid hormone plays an important role. In the absence of this hormone in the infantile organism whose pituitary gland is intact, there develops the stunted, or growth inhibited, form we know as the cretin.

Pituitary secretions likewise are essential for the growth, development, and function of other organs of internal secretion that play their essential and individual roles. Thus, in the absence of secretions from the pituitary gland, there is lack of growth and function on the part of the testis, ovary, adrenal, or thyroid gland.

ANDROGENS AND NORMAL GROWTH

The hormones of the testis, or the androgens in general, appear to have only little association with general somatic growth, but to exert a pronounced effect on genital growth. Not infrequently in the eunuch, there may be disco-ordinated growth, such as disproportionate increase in length of limb structure and general height; in other cases, however, the eunuch may be short and stocky. Other factors than merely the androgens appear largely

responsible, such as interactions with the pituitary as well as constitutional factors. In laboratory or domestic animals, castration exerts little influence on general growth, although in some cases, owing to lowering of general body activity, fat deposition may more readily occur.

Genital growth, on the other hand, shows positive correlation with androgens: they are largely responsible for growth of the various elements of the reproductive system, such as the epididymides, the vasa deferentia, the seminal vesicles, Cowper's and the prostate glands, the penis, and many secondary sex characters such as comb and wattles of birds, some aspects of horn growth, deer antlers, genital hair, and still other characters. The development and growth of these structures occurs not only in the young male approaching puberty and sexual maturity, but it is also a seasonal event in mammals with annual reproductive cycles. In the majority of mammals, an active reproductive system annually undergoes an extreme involution to be followed at the next season by its regrowth. Changes in the accessory reproductive organs may be of the order of 900 to 1300 per cent increase in fresh weight from the reproductive low; testis-weight increases may be as great as 500 to 1500 per cent. These changes depend upon hormones—testis increase upon pituitary hormones, and accessory-reproductive-organ increases upon androgens—hence it is obvious in such cases that hormone secretion is subject to periodic fluctuations.

Growth and development of the testis itself is largely dependent upon gonadal stimulating secretions from the anterior pituitary gland, as is evident from the data on pituitary ablation, replacement therapy, or certain dietary conditions that affect pituitary secretion. Growth of the accessory reproductive organs and secondary sexual characters depend upon the release into the organism of androgenic substances issuing in the main, if not wholly, from the testicle.

Since these circulating and growth-inciting substances pervade the entire organism, it is recognized that the resultant effects depend as much upon the peculiar capability of the end organ to respond to the hormone, as upon the stimulating capacity of

the substances themselves. We are largely in ignorance of the special peculiarities of an organ permitting it to respond to hormonal substances, or in fact, of the manner in which a hormone imparts its stimulating effect. It is becoming more and more apparent that enzyme activities are involved and one of our current problems is to learn the interdependence between enzyme action, the secretion of hormone by the organ of its origin, and the effects of the hormone within the organism.

It is a well-known fact that the increase in size and activity on the part of accessory reproductive organs involves rapid cell divisions, increases in cell size, and the initiation of secretory activity. These responses may be precipitous and express themselves within a week by weight increases of hundreds of per cent. However, although the responses are rapid, they are yet under control of natural regulatory mechanisms, contrary to rapid uncontrolled cellular activity in neoplastic development.

The period in life when the end organs of response are sensitive to hormonal stimulation is largely all pervading. Early in embryonic development, accessory organs of reproduction will respond to hormones in some animals; species variability is quite marked in this respect. These age responses on the part of the end organs are more definitely known than the period at which the naturally occurring hormones are first secreted. Introduction of androgens has been shown to stimulate the formation of certain end organs precipitously—notably the prostate gland—and induction of organ formation where it would not otherwise occur has been demonstrated. After formation, the various sensitive end organs are capable of responding to introduced hormone thus revealing the lack of sufficient high titers of hormone produced by the embryo itself. Mitotic activity and active growth are tremendously stimulated in the male organism by androgens but responses are not restricted to the male components; the uterus in the embryonic female, for example, is tremendously stimulated to increased mitotic activity and general growth, and the same applies to the other accessory reproductive organs and the phallus; the latter may be transformed into a characteristic penis in developing females within a short period of treatment. After forma-

tion and complete differentiation, as at puberty, it appears that the end organs are capable of response to introduced androgens as long as normal tissue is present; no one has demonstrated lack of response to androgens by an end organ that is due to age alone.

The relation of these normal growth phenomena, incited by androgenic substances, to the problem of neoplastic development are none too well indicated. The well-known testicular neoplasms, such as chorioepithelioma, are accompanied by unusual hormonal conditions in some cases; the blood and urine of such patients have an unusually high titer of gonad-stimulating substance, but it is not clear that this involves excessive androgen secretion. Certain cases demonstrate instead unusual quantities of estrogens sufficient to promote pronounced breast development and lactation. It is presumed that some effects emanate from the pituitary gland, but this is less well demonstrated and whether the condition could develop, or could continue to persist, after removal of the pituitary is not clear. The deranged tissue may possibly have attained a degree of independence that permits of an autonomous existence and deranged function. Whether pituitary secretions forward the activity of such testicular neoplasms has not been clearly demonstrated, although it is easily presumed such may be the case.

ANDROGENS AND NEOPLASTIC CONDITIONS

The relation of androgens to the development of neoplastic conditions in any organ is a current, unsettled problem. Some evidence exists that such a relationship holds. Prostatic cancer is perhaps the most frequently occurring among the genital cancers in the male and it is said never to occur in the eunuch and to be of low incidence in the Chinese. In the eunuch, the prostate is inactive and undeveloped owing to absence of androgens and suspicion is therefore directed toward an androgenic effect. In the Chinese, there is no known reason to attribute a low incidence to a lowered androgen secretion, since the Chinese appear to be too prolific for their own good. R. A. Moore and Melchionna have reported the production of squamous-cell carcinoma in rats by means of 1:2-benzpyrene, but they maintain that androgens play

no role in this production, since castrates develop it as well as does the normal male.

When prostatic carcinoma has developed, Huggins has convincingly demonstrated that activity, particularly of metastases, is markedly influenced by androgens. Definite clinical improvement in a high percentage of cases follows removal of the testes or the administration of estrogens; the latter treatment is presumed to inactivate pituitary stimulation of the testis thus removing androgen secretion, but it is possible that the estrogenic substance may also have a direct and inhibitory effect upon the atypical prostatic activity. Huggins has also demonstrated that administration of androgens in cases of prostatic carcinoma definitely intensified the disorder. Recurrences after castration, or lack of effect from castration in some patients, is believed to indicate a possible androgen secretion from the adrenal cortex.

The manner of androgenic influence upon these prostatic disorders, or the manner of influence of any hormone on an end organ, is not well understood. The studies of Huggins and co-workers on phosphatases in these human disorders, as well as the studies of Dempsey, Greep, and Wendler on the rat, strongly suggest that the solution of these problems will rest upon a further penetration into the subject of enzyme activity as an intermediary between the hormone and the end-organ response.

FACTORS INFLUENCING ENDOMETRIAL GROWTH IN MONKEYS, MACACA MULATTA

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The importance of this problem is such that much research has been done on it both in the laboratory and in the clinic. Methods and interpretations of data have differed, but it is agreed that the principal factors responsible for regulating growth and development of the endometrium are the ovarian hormones, estrogen and progesterone. Also, most investigators believe that the explanation of normal growth phenomena in the endometrium, as well as most pathology, lies in an understanding of the mode of action of these two ovarian substances. I should like to discuss here a series of experiments on one aspect of the general problem of the physiology of the endometrium, namely, the growth-promoting action of estrogen and progesterone.

ESTROGEN

After castration atrophy, all of the morphological characteristics of a normal endometrium present during the follicular phase of the menstrual cycle can be restored by the injection of estrogen. This can be accomplished in castrated monkeys within two or three weeks by giving a daily injection of a rather small dose of hormone, but to maintain the endometrium for any extended period requires a dosage that is above a rather specific threshold amount.[1] When the dosage of estrogen is small, uterine bleeding may occur if treatment be continued beyond thirty or forty days. When large amounts of estrogen are given, bleeding does not take

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place, even though the injections are continued for a year or more. Zuckerman found that the amount of estrogen required to maintain such endometrium for forty days without bleeding was about 200 I.U. daily; 10 γ alpha estradiol daily, equivalent to about 1000 I.U., was used in the experiments reported here. This is well above the minimal requirements for the prevention of bleeding during treatment; nor was menstruation observed in the animals, although in some instances, the injections were continued without interruption for many months. It is felt that 10 γ alpha estradiol is not an excessive amount and is well within the range of a normal physiological dose. The chief point of interest is the growth reaction of the endometrium as a function of time to a maintenance dosage of estrogen.

Engle and Smith[3] found that in castrated monkeys on estrogen for 100 days or longer, the endometrium was thinner than in animals on it for a much shorter time. On prolonged treatment, the endometrium became dense and the lumen small, although the size of the uterus remained about the same: in four animals, the only well-developed fundus was in the one on the shortest treatment, i.e., sixty days.

Hisaw[7] made similar observations and also reported that one of the initial responses of the endometrium to estrogen was a marked increase in mitotic activity in the glandular epithelium, which began to subside at about thirty days. During this time, the endometrium seemed to attain its maximal thickness. Cell division became progressively less frequent as the treatment was continued, and after 100 days, very few could be found. He also noticed a decrease in the thickness of the endometrium and ventured the opinion that the tissue, lost as a result of the precipitation of bleeding induced by lowering the dosage of estrogen during a chronic treatment, was not restored when injections of estrogen were resumed at the original level.

These results indicate that the ability of the endometrium of the castrated monkey to grow in response to estrogen stimulation is limited to a point beyond which a further increase in thickness does not occur. In fact, the maximal response cannot be maintained and a decrease in thickness cannot be prevented by continuing

treatment. The physiological and clinical implications of these observations are obvious, hence an attempt has been made to explore the reaction more fully.

Certain aspects of previous work were repeated with the present study in mind. In four young adult monkeys the uteri were brought into castration atrophy, then each was given a daily, subcutaneous injection of 10 γ alpha estradiol for ten, thirty, forty-five, and sixty days respectively. Eight hours before the end of the experiment each was given 3 mg. colchicine intraperitoneally. The reproductive tracts were removed and fixed in Bouin's fluid.

Each uterus was sectioned through its greatest diameter at the fallopian-tube level and the mitotic figures counted in measured areas of uterine epithelium lining the lumen in order to compare the number of mitoses per unit area of epithelium. This epithelium was chosen since it seemed satisfactory and offered fewer difficulties in making accurate measurements. Since it is one cell thick, but the height of the cells may vary, it was thought the data would be more significant if the comparisons were made in terms of surface area rather than tissue volume.

The mitotic activity of the animal given estrogen for ten days was very much greater than that found in the other three; between the tenth and the thirtieth days, the rate fell from 1751 mitoses to 688 per sq. cm. Then there was a further, but slower, decline to 516 per sq. cm. at forty-five days, and it remained the same (508 per sq. cm.) at sixty days.

These mitotic counts indicate that growth of the endometrium in response to estrogen is most rapid during the first two or three weeks of treatment. The endometrium also attained its maximal thickness during this time and, in fact, decreased in thickness if the treatment was continued for several weeks or months. Thus it seems that the endometrium passes through an initial period of rapid growth after which its responsiveness to estrogen declines.

It seemed possible that the musculature of the uterus might restrict the growth of the endometrium because of an estrogen-induced increase in tonus. In an attempt to remove any such influence, the uterus in two monkeys that had been given 10 γ

estradiol daily for twenty-one and seven days respectively was incised sagittally from fundus to cervix, the anterior wall denuded of endometrium, the incision left open in one and a wire loop sutured into it in the second, and the uteri returned to the abdomen. Judging by the mitotic activity, the endometrium of the second animal had arrived at or was approaching the period of its most active growth response to estrogen at the time the uterus was opened. The monkeys were continued on 30 γ estrogen for forty and twenty days. At the conclusion of the experiment, the uterus was completely repaired in both.

These observations suggest that the extent to which growth can be induced in the endometrium of a castrated monkey by the administration of estrogen is restricted to quite definite limits. However, before discussing this point further, it may be helpful to consider certain other experiments, particularly those dealing with the growth-promoting action of progesterone.

PROGESTERONE AND COMBINATIONS OF ESTROGEN AND PROGESTERONE

It had been found[4, 7, 9] that when castrated monkeys were given estrogen and progesterone simultaneously, the uterus attained a larger size and the endometrium was thicker than when the hormones were given separately in comparable doses for a similar period. It was thought that this synergistic action of the two ovarian hormones might be useful in obtaining additional information regarding endometrial growth in the incised uterus.

Two juvenile monkeys were castrated and given 10 γ estradiol daily for twenty days, followed immediately by 30 γ estradiol and 1 mg. progesterone daily for eighteen days. The uterus was then opened by an anterior sagittal incision and part of the endometrium of the anterior wall excised. The same dosage of estrogen and progesterone was then continued for twenty more days.

When the reproductive tracts were removed, an endometrial mass approximately the size of the uterus was found protruding through the incision. It had maintained the general morphology of the endometrium despite obvious distortions. Most of the extension beyond the lips of the incision seemed to correspond to

the functionalis. The glands of this region were drawn out into narrow, thin-walled tubes, whereas in the basalis they were greatly enlarged with elaborate papillary infolding of the glandular epithelium. The vascular system of the endometrium had also kept pace with the enormous outgrowth. The spiral arteries were "unwound" and could be seen as straight or loosely coiled vessels traversing two thirds or three fourths the distance toward the outer margin of the outgrowth.

This synergistic reaction of estrogen and progesterone is emphasized further by the action of progesterone when given alone. After castration and pretreatment with estrogen, a monkey was given 1 mg. of progesterone daily for eighteen days, the uterus opened as before, and the treatment continued for another twenty days. No endometrial outgrowth was found; in fact, the endometrium was thin and atrophic.

This experiment was repeated on two other monkeys, except that their uteri were opened at the conclusion of a twenty-day pretreatment with estrogen, after which 4 mg. progesterone was given daily—to one for twenty days, to the other for twenty-six days. There was a small outgrowth of endometrium in the first, but not in the other, although an excellent progestational reaction was present.

These observations clearly demonstrate that the combined action of estrogen and progesterone has a stronger growth-promoting effect on the endometrium than that furnished by the same amount of either hormone when given separately. In addition, these experiments raise a question with regard to the limitations of endometrial growth under these conditions. One may wonder whether it can be continued indefinitely or whether in time the endometrium may become refractory, as it seems to do with estrogen, and involute in spite of the continued action of the two hormones. Some information regarding the duration of responsiveness in an intact uterus was obtained from experiments in which estrogen and progesterone were injected concurrently for different periods of time.

An adult monkey was castrated and daily doses of 10 γ estradiol and 1 mg. progesterone started immediately and con-

tinued without interruption for 100 days. It is known that this combination of the two hormones is capable of producing a large uterus with a thick, fully developed, progestational endometrium within about twenty days. However, at 100 days, the endometria were astonishingly different from those of animals on similar experiments of shorter duration, being quite thin and the stroma dense. The glands were narrow, nonsecretory, and in the basalis reduced to cords of cells without lumina. In the stroma along the myoendometrial border, there were dense areas in which the cells were packed closely into clumps and cords. The origin of these structures is uncertain but their appearance and distribution suggest that they may have been derived from a cytomorphosis of the blood vessels. The general condition suggests involution rather than growth.

These experiments show conclusively that the endometria of both intact and incised uteri grow more extensively when both estrogen and progesterone are given. There is also, in the intact uterus as with estrogen alone, an initial period of rapid growth followed by refractoriness and involution. In the experiments on the incised uteri, the hormones were injected for a comparatively short period so it was not determined how long the endometrium would continue to grow under such conditions nor whether the large endometrial outgrowths would finally regress during a chronic treatment.

Uteroabdominal Fistulae

The experiments that have been described present the uncertainties inherent in most studies in which conclusions must be based on comparisons of material obtained from several different animals. Obviously, it would be more satisfactory if the response of an individual intact endometrium could be observed following the injection of estrogen and progesterone. An attempt was made to meet this condition by studying the growth of endometrium in uteroabdominal fistulae. The surgical technique used was patterned after that already described.[12, 13] It was found that the fistulae could be maintained in good condition for an indefinite period.

Each monkey was started on 10 γ estradiol daily immediately after recovery from the operation. The fistulae were such that it was possible to make frequent inspections, either by hand lens or dissecting microscope, of most of the upper part of the endometrium on the anterior and posterior walls of the uterus. One monkey was kept on estrogen for fourteen days, another for twenty-two days; at no time did the endometrium of either show any tendency whatever to grow out through the fistular opening. The elliptical slit formed by the endometrium of the two opposing walls could be located easily, the two sides pressed apart by any small, smooth instrument and the surface of the endometrium examined. Changes in thickness of the endometrium could not be ascertained without resorting to biopsies, which it was feared would disrupt the experiments, but the impression gained was that after a time, there was little or no growth as indicated by lack in change in general appearance. The limited growth observed in these experiments is in agreement with that obtained with estrogen on intact and incised uteri.

A very remarkable change occurred in the endometria when 1 or 2 mg. progesterone daily was added to the treatment. Endometrium that had apparently reached its maximal response to estrogen showed active growth almost immediately following the injections of the two hormones. By the fourth or fifth day, lobes of blood-red endometrium began to protrude through the openings of the fistulae. Within a few days, tonguelike processes of endometrial tissue were thrust out of the opening with each uterine contraction and entirely or partially withdrawn at each relaxation.

It was difficult to protect these outgrowths against mechanical injury and consequent tissue loss, but when successful, the identity of the individual lobes could be maintained and studied for several consecutive days. Occasionally, one or more of the lobes became ischemic, probably as a result of crowding and mechanical constriction of their blood vessels. These stood out in sharp contrast among their richly vascularized neighbors. They were apparently devoid of a blood supply, at least in their outer extremities, since surgical removal did not result in bleeding and

histological examination showed considerable thrombosis. Yet if undisturbed, they often remained for several days and were finally lost by what seemed to be a process of crumbling away of their tissues.

It was also possible to precipitate menstruation. In one of these experiments, the animal was first on estrogen for twenty-two days, during which time the endometrium did not grow sufficiently to obstruct examination of the walls of the uterus. Periodic blushing and blanching of the endometrium could be seen, but there was always the possibility that this might have been due to mechanical stimulation. Beginning on the twenty-third day, 2 mg. progesterone was added to the daily treatment. The first effect of progesterone was an increase in the color of the endometrium, which became noticeable toward the end of the second day. On the fourth day, a small amount of stringy mucus was present in the lumen and there appeared to be definite growth. Growth of the endometrium became obvious on the fifth day. A lobe of endometrium projected into the mouth of the fistula on the sixth, and it was completely closed by a small plug of tissue on the seventh. Rapid growth and protrusion of the endometrium through the fistula continued and was associated with the usual loss of tissue as a result of strangulation and mechanical injury.

The injections of progesterone were stopped after eleven days, and the animal continued on 10 γ estradiol daily. No change in the endometrium was noticed on the first day following withdrawal, but by the second, the difference was spectacular: the extruded endometrium had been withdrawn into the uterus, the mouth of the fistula was open, and one could look down into the uterus. The endometrium was ischemic and there was no active bleeding. Menstruation started during the night of the second day and continued two days.

The rapid disappearance of the endometrial outgrowth into the uterus following progesterone withdrawal was strikingly like that described by van Wagenen and Morse as occurring shortly before menstruation in the normal cycles of monkeys with uteroabdominal fistulae. The ischemia of the endometrium on the anterior and posterior walls of the uterus was doubtless comparable to that

associated with premenstrual collapse as seen by Markee in endometrial implants in the anterior chamber of the eye.

The extraordinary growth potential shown by the endometrium in uteroabdominal fistulae raised the question as to how long it would continue when subjected to the continuous stimulation of estrogen and progesterone. It has been mentioned that the intact uterus seems to become refractory and in time shows involutionary changes. Whether this also would be true of an endometrium under conditions in which growth was unobstructed seemed worth considering.

One uteroabdominal-fistula monkey was given 10 γ estradiol and 2 mg. progesterone daily for ninety-eight days. The fistula was examined daily and the amount and character of the outgrowth was recorded as written descriptions, diagrams, and occasional photographs; the unavoidable loss of tissue made impossible a more quantitative estimate of the amount of endometrium produced. An idea of the rate of growth was obtained by periodically removing all of the tissue that protruded through the fistula and noting the time required for a new outgrowth to develop. It was found that the endometrium was still capable of growing at ninety-eight days, but the rate seemed considerably slower than at the beginning of the experiment. Also, the total amount of endometrial tissue produced was more than that present at any given time in the intact uterus of two animals on similar treatment, in one of which the endometrium had become atrophic at the hundredth day. Hence, a combination of estrogen and progesterone can maintain growth in a uteroabdominal fistula for a much longer time than in the intact uterus.

These observations on uteroabdominal fistulae furnish additional evidence that the limited growth of the endometrium in response to estrogen is not due to any influence exercised by the uterine muscle. It seems more likely that the unresponsive condition attained during a chronic treatment with estrogen represents a physiological property of the endometrium itself. Whether this is also true when both estrogen and progesterone are given is still open to question, since the endometrium in this situation continued to grow out of the fistula for at least one hundred days,

whereas, the endometrium of a normal uterus shows marked involutionary changes by this time.

The Exteriorized Uterus

Uteroabdominal fistulae offer several obvious advantages for studying the endometrium, the most important being the accessibility for making direct observations and obtaining biopsy material. They also have certain limitations. Among the most serious of these is the encroachment of the skin on the mouth of the fistula. Also, when the fistula is closed by a plug of endometrial tissue, it is not possible to determine by direct observation what regions of the endometrium are contributing tissue to the outgrowth. It was with this last difficulty that we became particularly concerned.

It was thought that if a differential growth of areas of the endometrium occurred, it probably could be determined by a preparation in which the growth response of the whole endometrium could be observed. The uterus was exteriorized and divided transversely, and the anterior half everted and deflected downward in an operation very similar to that used in making a uteroabdominal fistula.

The endometrium seemed to retain its normal condition for at least the first few days after the uterus was opened. When examined under a dissecting microscope, small localized areas of transient ischemia could be seen, probably marking the positions of the coiled arteries. Also there was a periodic general blanching associated with the rhythmic contractions of the muscularis. These observations are in general agreement with what one should expect,[11] but did not seem true of the whole endometrium, for a zone surrounding the internal os tended to retain its blood-red color even during strong contractions of the uterus.

Another difference between these two regions of the endometrium was seen in their ability to survive in the exteriorized uterus. That of the anterior and posterior walls gradually deteriorated despite the best attention; possible infection did not seem to be the most important factor determining survival—exposure to occasional drying and mechanical irritation were probably more im-

portant. Yet, even when these problems were apparently solved, the surface of the endometrium underwent disorganization associated with thrombosis of superficial blood vessels and loss of tissue without bleeding.

The reaction of the endometrium at the cervix was in sharp contrast with this. It did not degenerate, but retained its capacity to grow, and has been maintained in good condition in one uterus for nine months. Consequently, the endometrium at the cervical end of the uterus was of special interest for further studies.

This difference between the endometrium of fundus and cervix became more pronounced under the influence of the ovarian hormones. At the cervix it grew rapidly and within a few days stood out as large elliptical lips surrounding the internal os. They were sharply separated from the rest of the endometrium by a deep groove around their outer borders. The distinctiveness of their tissue was also emphasized by the fact that it did not take part in the rhythmic blushing and blanching of the uterus. Even when the two lobes of the incised uterus were thrown into strong contractions by mechanical stimulation, the lips retained their blood-red color in contrast with the general ischemia.

The growth of the endometrium of the lower segment seemed to depend on the same physiological factors as that of other parts of the uterus. The response to estrogen was slower and less extensive than to a combination of estrogen and progesterone. It was also capable of menstruation. The most striking peculiarity observed was its failure to take part in the rhythmic vascular changes. This led to a number of experiments on growth and menstruation as seen in this tissue. One of these was the precipitation of menstruation in the presence of estrogen by injections of progesterone. A monkey was given 10 γ estradiol daily for fifteen days during which time the cervical lips of the endometrium became well developed; 1 mg. of progesterone, in addition to estrogen, was given on the sixteenth and seventeenth days, after which the animal was continued on estrogen alone. On the afternoon of the third day (the twentieth of the experiment), a small hemorrhagic area was found on the endometrial lip of the posterior wall. The blood did not form a hematoma; it was interstitial

and some was seeping into the uterine lumen. This condition had spread to both lips by the next day, and on the following morning (the twenty-second), all the cervical endometrium was involved in active menstruation. Bleeding continued through the twenty-third day, and by the twenty-fourth, both lips of the endometrium were entirely gone and no blood was present in the uterine lumen.

The repair of the endometrium and the initiation of new growth following menstruation was astonishingly rapid. Active bleeding and loss of endometrial tissue occurred on the fifth and sixth days after the last injection of progesterone. A surface epithelium was apparently restored to the denuded areas within two days, and within five days after menstruation, two prominent ridges could be seen marking the position of two new endometrial lips.

The accessibility of the endometrium also made it possible to test the effects of progesterone on menstruation when applied topically. Thus, a monkey was given 10 γ estradiol daily for twenty-three days, by which time the cervical endometrial lips were well developed. On the twenty-fourth day, the lips were parted, and 2 γ progesterone in 0.02 ml. sesame oil dropped into the lumen and spread over the lips by manipulating the uterus. The injections of estrogen were continued without interruption.

The progesterone was applied at 3 P.M. and by noon of the third day (about fifty-seven hours) the color of the endometrium had become considerably darker, actually almost purple. This color was retained even when strong contractions of the uterine muscle were induced by rubbing it gently with a swab. The condition of the endometrial tissue seemed to be the same as that following the subcutaneous injection of 2 mg. progesterone as already described. Menstruation started on the fourth day and bleeding had stopped by noon of the sixth. The endometrial lips were entirely lost. As control, 0.02 ml. sesame oil alone was without effect on the endometrium, but a repetition of 2 γ progesterone in 2 ml. sesame oil produced the already described effects.

The most important result of these experiments is the difference brought out between the endometrium of the fundus and that surrounding the internal os. The absence of rhythmic vascular changes in the endometrium at the cervix and its failure to

undergo ischemia either before or during menstruation seem most significant. Studies of biopsies and sagittal sections of the lower segment of the uterus indicated that the endometrial lips seen in the exteriorized uterus were derived from an area not provided with coiled arteries.

DISCUSSION

The results of these experiments on monkeys may contribute to a better understanding of the factors involved in endometrial growth, but the problems they suggest may be of greater importance. The limited response of the endometrium to estrogen is a case in point. The atrophic endometrium of a castrated animal receiving estrogen attains its maximal thickness in two or three weeks and this is not maintained when the treatment is continued but, in time, the endometrium actually becomes thinner. Also, once an endometrium becomes unresponsive to estrogen, it apparently remains so as long as it is under the influence of the hormone. These changes seem related more closely to the duration of treatment than to the dosage of hormone given. Hartman (personal communication) obtained similar results when the amount of estrogen given was many times that used in the experiments reported here.

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V

Newer Concepts in the Chemistry of Growth

MODE OF ACTION OF THE NITROGEN MUSTARDS—A NEW WORKING HYPOTHESIS AND ITS POSSIBLE RELATION TO CARCINOGENESIS

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At the outset of this lecture, I should like to make two points clear. First, the views that I wish to put forward must be regarded, for the present at least, as constituting what is very strictly a working hypothesis alone. As such, they may not, I trust, prove altogether unsuited to the present occasion, when it should perhaps be part of our task to consider new ideas and trends of thought, in contradistinction to work that is already recorded and accepted or essentially complete. In the second place, this working hypothesis is one that has emerged through the fusion of initially independent contributions made by various individual members of the staff of the Chester Beatty Research Institute, in particular Ross, Goldacre, and Loveless in the chemical, physicochemical, and cytochemical fields respectively. Very considerable assistance has also been given from the cytological, pathological, and biochemical aspects by Koller, Horning, and Elson. I should therefore be regarded simply as a spokesman on behalf of these various workers, and as endeavouring to the best of my ability to bring together the different threads of their contributions, while accepting full responsibility for the way in which this is done.

As has recently been described in a paper by Kon, Harris, Roe,

and myself,[2] we discovered some time ago the biological properties of growth-inhibitory power and carcinogenicity, which had previously been known to be associated in the case of the carcinogenic hydrocarbons, in 4-aminostilbene and a number of its derivatives, which can therefore be regarded as constituting a group of tumour-producing substances of an entirely new chemical type. In the course of this investigation, we were led, in varying the substituents attached to the nitrogen atom, to examine the compounds in which these substituents are di(β-chloroethyl) groups as in the nitrogen mustards. In their biological effects, the new compounds proved relatively uninteresting, but we then proceeded in turn to investigate in what other types of aromatic amine, if any, such di(β-chloroethyl) groups might still exert their characteristic cytotoxic effects.[1] At once it became apparent that such cytotoxic properties are in fact a feature of the di(β-chloroethyl) derivatives of such aromatic amines as aniline, *p*-aminobenzoic acid, and β-naphthylamine, to mention only a few of the long series that has now been examined. These substances are indeed very strongly radiomimetic, as is shown, for example, by their capacity to induce greying of hair on subcutaneous injection in the mouse, an effect that can equally be induced by roentgen-ray radiation, by injection of the aliphatic nitrogen mustards as shown by Boyland, or by the subcutaneous injection of a radioactive element such as plutonium. Such radiomimetic qualities are also reflected in the therapeutic effects brought about by these substances in Hodgkin's disease and chronic leukaemia, by the undoubted depressant effect of these compounds on the normal bone marrow, and by damage to other tissues in a state of active cell division such as the testis. Thus, degenerate structures are seen in the undifferentiated spermatocytes as early as six days following administration of di(2-chloroethyl)aniline in the rat, while an extreme degree of atrophy is produced by the same treatment after thirty days. The radiomimetic property is even more strikingly shown in the acute cytological effects of these compounds, the most frequent abnormalities being chromosome bridges at anaphase, chromosome fragmentation, lack of chromosome spiralisation, and the

ejection of fragmented material from the nucleus in the form of micronuclei.

Let us now turn to consider the possible mode of action of these aromatic mustards, and the relation between their chemical constitution and biological action. From examination of some 150 such compounds, it would appear that the outstanding requirements are (a) the presence of at least two halogenoalkyl groups in the molecule, and (b) a certain minimum reactivity of the halogens. The need for two such reactive side-chains is based upon the biological examination of various pairs of compounds, one possessing two side-chains and the other one, and I wish to draw special attention to this requirement for cytotoxic activity, since it is this feature more than any other that the present hypothesis attempts to explain. At an early stage, but with no special application to this particular problem at that time, Goldacre set out to study the physicochemical mechanism of adsorption of various drugs to proteins, bearing in mind the special case of mitotic poisons, and the possible interpretation of the mitotic cycle as a reversible naturation-denaturation or coiling and uncoiling of protein fibres. From this emerged the general conception that adsorption of certain cytotoxic compounds might well occur to the fibre in its extended phase, in such a way as might introduce steric and other impediments to subsequent re-contraction.These views provided a remarkable parallel to conclusions that were independently being drawn from purely cytological considerations, namely, that the initial effects of the nitrogen mustards might well be produced in the resting stage, their incompatibility with normal mitosis only being revealed (by bridge formation and fragmentation) in the actual course of cell division, and being augmented as one abnormal division succeeded another, eventually leading to death of the cell. Ross was meantime considering the kinetics of hydrolysis of the aromatic mustards, and the possibility that the two requisite side-chains might react with two independent and suitably spaced receptors lying either on a single surface or fibre or on two contiguous fibres. Thus, if the first haloalkylamino group becomes anchored to a reactive centre, the second group may approach and react

with another centre either on the same protein chain or on an adjacent one. Of all the various possibilities, the actual process of cross-linkage between separate fibres is of particular interest, and a specially favourable opportunity for its formation occurs just before mitosis: during interphase the gene string is probably of only molecular dimensions, and it is likely that on reduplication or splitting the sister chromatid fibres are only of the order of chemical bond distance apart and are identical—a situation which may well be unique in the cell. The cytological abnormalities, namely, chromosome fragmentation, bridge formation at anaphase, and, as an early effect, "stickiness," which are such a prominent feature of the action of the mustards, would thus acquire a new explanation. On this hypothesis, "stickiness" is seen as the consequence of cross-linkage between chromosomes already in mitosis, which does not normally lead to breakage, and fragmentation as the result either of tension upon the extended threads of the interphase chromosomes cross-linked to produce a junction stronger than the fibres themselves, or of rupture in what can be regarded as an extended polymer. A chromosome segment thus produced, which retains the centromere and has broken distally to the cross-linkage, would produce a bridge at anaphase, and such an explanation would account for the events that have been generally interpreted as due to breakage followed by reunion of broken ends. As has been pointed out, the cytological changes due to the mustards are remarkably similar to those observed after treatment by roentgen-ray radiation, and in the latter case equally, it would appear worthy of investigation whether the effects are due to cross-linkage between adjacent protein chains. Such a union could arise, for example, by the interaction of two sulphydryl groups under the oxidative influence of roentgen-ray radiation, which is known to occur in vitro.

Consideration of the above principles has suggested that many other molecular types, containing two or more groupings capable of similar reaction with functional centres in biological systems, would be effective as cytotoxic agents. Examples are already known of active compounds in which the two requisite haloethyl groups are attached to different nitrogen atoms, and it is obvious

that a great number of variants, especially involving heterocyclic types and modification of the side chains, calls for investigation; work along these lines is proceeding.

We now come to a fascinating extension of these possibilities, namely, their relation to the process of carcinogenesis. I have elsewhere referred to the discovery made some time ago by Elson: that the characteristic growth-inhibitory action of the carcinogenic hydrocarbons and aminostilbenes, which we regard as an essential prelude to their carcinogenic effect, can be greatly reduced by the incorporation in the diet of a sufficiently high proportion of protein. From comparison of the effects of the carcinogenic hydrocarbons, the carcinogenic aminostilbenes, and the aromatic mustards on the growth of various experimental tumours, it was at first believed that growth inhibition by the mustards, in contrast with that produced by the carcinogenic hydrocarbons and aminostilbenes, was probably independent of protein and due to some other mechanism. Closer investigation has, however, revealed that the response is in fact modified by protein, and that the apparent lack of such an effect in earlier experiments was rather due to the higher intensity of the inhibition caused by the mustards. We now regard these relationships as essentially a family of curves, reflecting the same type of dependence upon protein of growth inhibition whether produced by the carcinogenic hydrocarbons and aminostilbenes on the one hand, or by the nitrogen mustards on the other.

These results naturally raised the question of the potential carcinogenicity of the nitrogen mustards, and it is of great interest that Boyland and Horning had meantime obtained independent evidence of tumour induction in mice by means of repeated injection of the aliphatic mustards HN2 and HN3, while experiments to test the carcinogenicity of the aromatic mustards have now yielded positive evidence (Haddow, Horning, and Koller: in preparation) of sarcoma production at the site of injection of the di(2-chloroethyl) derivatives of α- and β-naphthylamine in the rat. Interesting as these tumours are with respect to the general theory of carcinogenesis, they acquire far greater significance in relation to the hypothesis of cross-linking under discussion, when we con-

sider the remarkable cytological abnormalities which many of them are now found to possess. Outstanding among these is a strikingly high incidence of bridges at anaphase, while multiple bridged anaphase groups and chromosome fragmentation are also observed. Not only is the incidence of such abnormality remarkably high—e.g., 60 to 70 per cent of bridges at anaphase—but it is maintained, apparently as a characteristic feature of such tumours, in subsequent transplanted generations.

It need not be stressed how such views and findings are likely to have a considerable impact upon many of our problems, not least in the long-postulated connexion between growth inhibition and carcinogenesis. In the case of the carcinogenic mustards may it be that the initial interference with growth is brought about by direct combination with the constituent chains of the chromosome fibre, and that the eventual emergence of a new cell strain is due to the subsequent and independent replication of such fibres genetically modified at the sites of such combination or cross-linkage? If so, is it feasible that carcinogens of other types may operate by some such similar mechanism? It is an extraordinary fact that we are still quite unaware of the significant site of combination of the carcinogenic hydrocarbons; while an immense amount of investigation still remains to be completed, there can be no doubt of the great impetus to future development that would follow some such clarification. A final problem, and one that has proved an insoluble puzzle for many years, lies in the fact that cytological abnormalities may be characteristically frequent in many individual tumours and apparently quite absent in others: is it possible that their striking abundance in some of the mustard-induced tumours is due to the production of mutations at a large number of loci simultaneously, and that this may in turn be due to the high chemical reactivity of this particular class of carcinogen?

In conclusion I should like to re-emphasize what I said at the beginning, namely, that the view presented is a working hypothesis only, which has still to be adequately tested and the chief merit of which may be the stimulus that it provides to further experiment and thought. If it is indeed true that chemical sub-

stances of these kinds produce some of their characteristic effects by reacting directly with the genetic determiners in the uncoiled resting chromosome in the manner described, an entirely new approach will certainly become available to the closely related problems of growth inhibition, induced mutation and carcinogenesis. Meantime the hypothesis is simple at any rate in principle, shows every sign of being of a very fertilising kind, and has already stimulated a great deal of work, the results of which may extend far beyond the problem of the mode of action of the nitrogen mustards.

A fuller account of these experiments is in course of preparation.

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Part Two

PRINCIPAL ADDRESSES

TIME AND THE CANCER PROBLEM

by KENNETH C. D. HICKMAN, PH.D.
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The invitation to make an address on this momentous occasion before persons of great national eminence and distinguished guests brought from all parts of the world was not to be accepted lightly, particularly as cancer is not my professional field. Some years ago, while planning a speaking tour in the West, I received a telegram from Oregon asking whether I would address a joint meeting of the Oregon Medical Society and the local section of the American Chemical Society in Portland; and in an extrovert moment I telegraphed, "Delighted to speak anywhere, any time, any subject." This telegram has lived to plague me as evidence of a desire to arise in public meetings. The pay-off came in Portland, for in the audience there was a Nobel prize winner and my chairman was so impressed that he introduced him before presenting me and then could not remember my name nor where I came from!

There is an old saying that "Journeys end in lovers' meetings." Transcribed into the idiom of science, we say, "Events occur at interfaces." Events only happen when unlike entities come into contact. The birth of a man or the birth of an idea spring from events at interfaces. This great meeting here in Memphis is an interface of a dozen—a hundred—facets where science and experience meet together to conquer cancer.

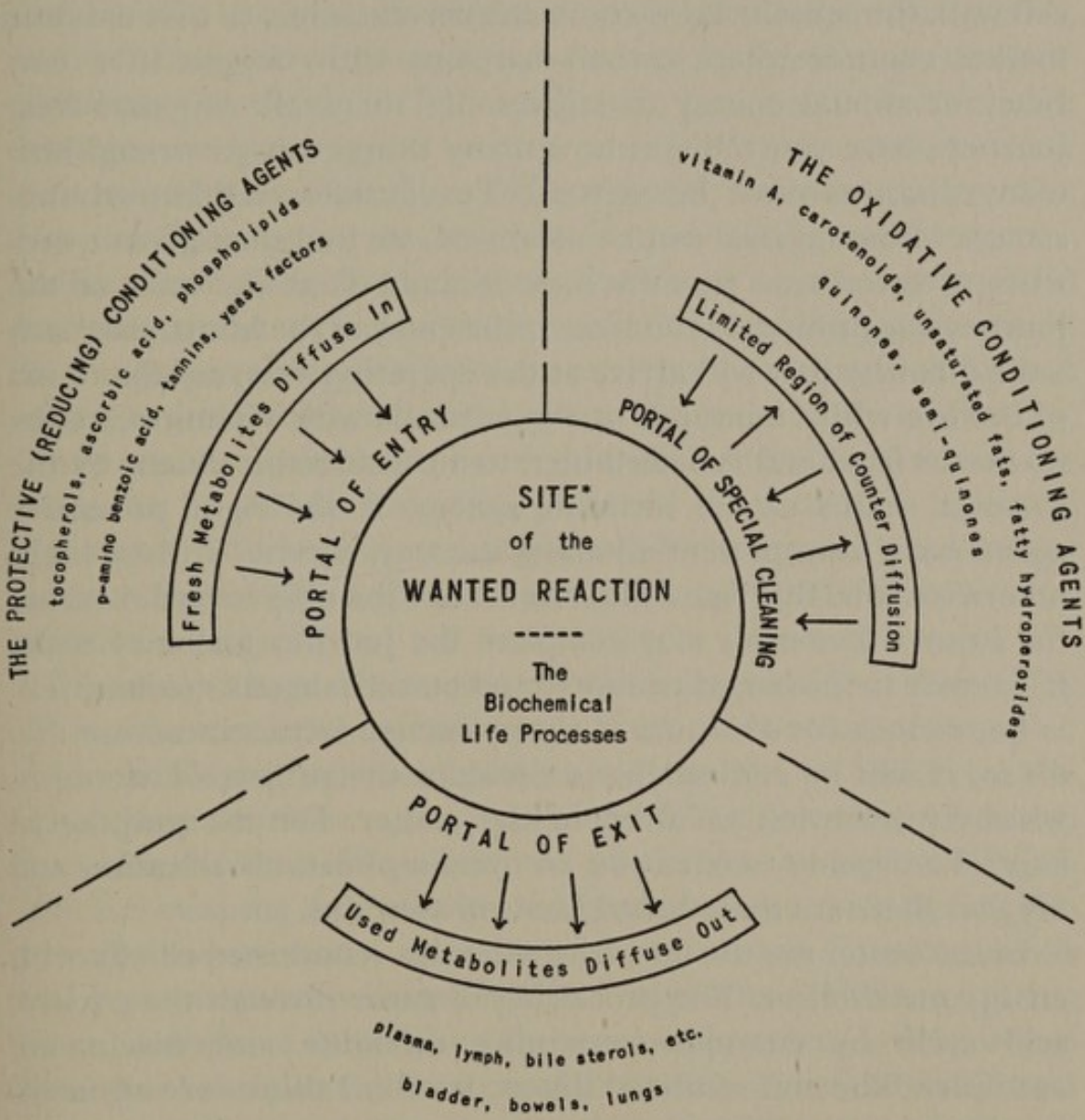
I am by profession a chemical engineer, by trade a vacuum plumber, and by avocation an amateur in biochemistry. I began to watch the cancer horizon about ten years ago in the belief that fat metabolism, and hence the technology of fats—in particular high-vacuum distillation and thus vacuum plumbing—would have a significant place in the foreground. I still believe so.

If we could make a broad generalization concerning the physician and the chemist, it would be that the doctor regards the

human body as a complicated machine whose parts can become mechanically deranged—against a background of standard and scarcely varying chemistry. The chemist, on the other hand, too often thinks of the body with its myriad of interlocking organisms as a standard machine whose chemistry he can vary widely while the machine functions unchanged. I shall confine myself to the latter incomplete point of view tonight.

Events occur at interfaces. The ultimate interfaces in the human body (forgive my untutored use of the term "body." You doctors refer to a body only as a *foreign body* that is removed from a human body. I shall be referring to the human body again and again tonight) are the enzymes that promote all the separate activities of living. These enzymes may be a few molecules in solution or they may be monolayers of active chemicals. Nutrient substances that are capable of reacting with the enzymes diffuse through the cell to the monolayer, "do their stuff" and then diffuse away again as altered products. Has it occurred to you that at any moment only a few molecules—perhaps one out of every million, or even billion—is in the act of making this encounter? All the rest are waiting to reach the site of action or are trying to leave again; or are forming part of the inactive structure—bones, blood vessels, capillaries, inert solution, and so on—necessary to house or transport the active fraction. We are in the habit of using two terms in biochemistry—in vivo, meaning in the living body and in vitro, meaning literally in the glass, or shall we say the experimental vessel. May I suggest to you that a third term, *in transitro,* or "on the journey," is required to denote that important condition of metabolic matter (and by far the most prevalent condition of all) in which the materials are waiting or in storage or on a journey to and from the site of activity? My appearance here tonight, where I stand at the interface of your informed public opinion, is the culmination of years of study, long weeks of preparation by your organizers, and a long tiresome journey to this spot, and having "done my stuff," the broken fragments of me will diffuse away home again. There is this important difference between my arrival and my leaving, namely that everyone was highly concerned with the process of getting me here and no

one cares what happens to me now. In our study of the metabolism of the human body, there is this same distortion of viewpoint—tremendous attention given to *how* materials reach the operating



* Note, this circle does not necessarily denote the cell as a whole, but rather a focus of metabolic activity within the cell.

interface and almost total disregard for what happens to them *afterward*. I shall suggest to you that what happens on the way out, and in particular, what happens to the fragments that cannot get out, may be at the very heart of the causative problem of cancer.

Going back to the journey in. In the Grand Sense this journey

is from the seed, through the growing plant, the harvest (or slaughter-house), the market place, the kitchen, the cookpot, the stomach, the intestinal wall, the circulation, the liver, the storage deposit, the channels of redistribution, the intracellular fluid, the cell wall, the cell, the fat vacuole, the chromosome, to that enzymic market counter where carbon bargains with oxygen in a currency of animal energy that gives life to us all. On that long journey—have you thought how many things can go wrong, how many disasters must be skirted? To consider well-known substances whose survival can be measured, we find that, given quantities of carotin or vitamin A or vitamin C at the start of the journey—beginning, if you like, at the entry of the human stomach —the quantity that will arrive at the operating sites, say the retina of the eye with vitamin A or the adrenals with vitamin C, is by no means fixed and is determined to a considerable extent by the chemical status of the intransit system. If the right protective agents have been present all along the way, survival will be high; otherwise, low. But lack of survival is not the only hazard, because the injured materials may complete the journey and may make false entry to the bargain counter and be exchanged as counterfeit.

Let us look for a minute at the protective factors in nature. To do so, it will be convenient to consider the subject of vitamins, which is somewhat involved in the matter. For the purpose in hand, I am going to offer you an oversimplified classification and say that there are three broad kinds of vitamins.

In the center are the operative vitamins—concerned chiefly with energy metabolism. The processing of sugar through the pyruvic acid cycle by enzymes containing thiamine and niacin are examples. The end results—I digest, I walk, I think—are all facilitated by the operative vitamins.

My second classification contains the structure vitamins—D for bone formation, A for cell differentiation, K for blood structure, and so on.

The third classification, the one of prime importance tonight and one, I may say, that has hitherto received insufficient recognition as such, contains the *conditioning* vitamins. Their action is secondary but oh, how tremendously important, for it is they that

control the fate of the metabolites, including the primary vitamins on their long and hazardous journeys to and from their sites of operation. This class consists of a number of interlocking individuals, sometimes opposing, sometimes co-operating with one another. On the one side—and by far the most important on this, the *reducing* side of the equation—are the E vitamins, particularly alpha-tocopherol aided by vitamin C, para-aminobenzoic acid, lecithin, the tannins, and certain yeast factors, to mention those we know about so far. They form together the great preservative complex that stops unwanted oxidation every way along the journey from the growing plant to the site of action in the animal and then to the very excrement from the bowel on the final exit. In a highly reactive environment—the body—surrounded by and saturated with oxygens, they prevent *unauthorized* combustion. They are the sprinkler system, the fire insurance agents of the body. As I view the longevity problem, it contains as a central question, "How much can we have of the preservative factors without depressing active metabolism, making the organism as a whole lethargic?"

These agents, as I have just mentioned, must be balanced by others of an exactly opposite kind. We have been so preoccupied with studies on sugar and protein metabolism in which we can watch large nutritive molecules being broken down atom by atom by a series of interrelated enzymes all the way to carbon dioxide, which is escaping, for instance, as a gas from my mouth during this speech, that we forget that there are substances like the sterols and hormones and perhaps endogenously formed *carcinogens* that must be disposed of continually because they cannot diffuse out, and one of the alternative ways of disposal is by oxidation. There is much evidence to suggest that there is a professional class of scavengers or incinerators of waste products in the body and that the class may contain vitamin A, the essential unsaturated fats, the fatty hydroperoxides, and the quinones and semiquinones of vitamin E and C.

Some very simple examples will illustrate this point. Dissolve some carotin in olive oil and pour it into four test tubes and keep it on your laboratory bench or office desk. Add nothing to test

tube No. 1. Add a few drops of cod-liver oil to No. 2, cod-liver oil plus vitamin E to No. 3, and vitamin E alone to No. 4. At the end of a week, all the test tubes appear the same except No. 2, which has lost its color. This has been caused by the vitamin A and essential fats in the cod-liver oil promoting the oxidation of carotin. Let us inspect the tubes again at the end of a month or a year. Now tubes Nos. 1 and 2 are colorless, but tubes 3 and 4 retain their carotin. The vitamin E has prevented oxidation even in the presence of the promoter, vitamin A.

That was an in vitro experiment. On my tour out West that led to Oregon, a professor at an agricultural college told me that if you feed enough vitamin A to a red Leghorn hen, you will bleach its feathers and turn it into an imitation Buff Orphington and the hen will lay an egg with a white yolk. The vitamin A has bleached the carotinoids without disturbing the rest of the metabolism of the hen. Now give vitamin E and the color of the feathers will come back, the egg yolk will turn dark, and the yolk will be found to contain the carotinoids as well as the added vitamin A and E living in a balanced harmony. This is an exact replica of the first experiment, but in vivo.

You will notice that I have cited vitamin A so far in three distinct roles, first, and of course begging the question by calling it the tissue-differentiating vitamin, in the *structural* class. Then by citing its conversion to retinene which is in a *functional* class, and finally by calling it a scavenger agent in the *conditioning* class.

This leads me to introduce a profound scientific concept—the "screwdriver" hypothesis—which as learned scientists and distinguished physicians you will find highly useful. You acquire a screwdriver in order to drive a particular screw, but you use it for all kinds of screws. Eventually you cannot lay your hand upon it because it has strayed to your wife's sewing machine and later to the kitchen for opening a soup can and finally it is bent by opening a jammed window. In short, wherever a screwdriver can be employed, or even prostituted, there it will be put to service. And so I think it is with biologically potent substances. The functions of each one may be few or legion, according to its versatility. We are perpetually forgetting this essential fact, and when we have found

one obvious function of a new metabolic agent, we are apt to say, "well, that's that," and close the books on our inquiry. This has a very stultifying effect on future research. I shall be referring to this matter again.

To return to our main theme; the great secondary activity of the body is the conveyance of metabolites to and from their place of operation. But whereas an immense amount of study has been given to the arrival, very little has been given to the exit of the after products. However, exactly as much waste material must escape as fresh material enters. Garbage disposal is every whit as important as city delivery, but it is not as fashionable.

During the past fifteen years, an ever-growing witch hunt has been made for carcinogens, those chemotherapeutic agents that induce cancer. Men are spending whole lives studying chemical after chemical that in ever-smaller quantities will produce with ever-greater certainty a tumor in a mouse. They remind me of my own cult of vacuum technologists who will proudly display a bottle that is emptier than has ever been secured before. With a slightly wider inquiry, the investigator asks what ingested chemical causes the normal cell to become an uncontrolled cancer cell. Is it overcooked fat or industrial dirt or smoke or foods cultivated from synthetically fertilized land; or is it just a statistical phenomenon of an increasingly aged population?

I want to suggest to you that cancer may be produced less often by a toxic principle getting into the body than by toxic by-products created in the body being unable to get out. Cancer in an aging population would thus be a symptom of accumulating internal dirt; of the cell becoming an ancient and dusty attic where all those fragments or elaborated substances that happen not to be able to escape have accumulated until the cell passes into a condition of revolt.

Let us examine this Time factor. It is a curious fact that with all the medicines of natural or artificial origin that have been administered over hundred of years, no mathematical calculation appears to have been made by the doctors concerning the time of accumulation in the human body or the time of depletion. To consider my old friends, the vitamins; supposing your bodies were

completely empty and the problem was to restore them with vitamins, giving each day the amount contained in the day's food, how long would it take to "fill an empty body"? We must know two things: first, the average daily content of the food, and second, the "normal" content of the body. If we perform this calculation for vitamin B_1, we shall find that there are about 60 mg. contained in all the tissues. The amount in the day's food is about 2 mg. So to fill our static living vessel, it would take thirty days. But the body is not static, it is dynamic. We are consuming vitamins at the same rate that we acquire them, a little up one day, a little down another, but in general there is a constant-level device operating in the body; so as soon as we start pouring thiamine into the empty body *some* thiamine will start to decompose or diffuse out. How long will it take to fill the body? The answer is, all eternity. So to be practical we ask how long will it take to make a *half change* in body status? Here the laws of washing of a photographic film or of the rate of decay of radioactive substance come into play and we can say that the time of half adjustment is the logarithm of 2 multiplied by the *dosage ratio,* the ratio between daily intake and the normal content of foods. The half period of thiamine is, thus, twenty-one days. The half period of vitamin D turns out to be only sixteen days but when we come to do the calculation for the in transitro conditioning vitamins, we find vitamin A with a half period of more than a hundred days and vitamin E with a half period anywhere from six months to four or five years! No wonder the doctors have been unable to discover what vitamin E does. It may take ten years to alter the vitamin-E status of a distant organ in the body. If, then, the conditioning agents themselves have these long periods of adjustment, how about the heavy, elaborated metabolites that are produced in small quantities all the time in the cell? I suggest to you that cancer, or rather a predisposition to a cancerous condition is a factor of time, not only as long as the life of an individual but perhaps extending back through generations!

If there is any branch of chemotherapy that is receiving more than ordinary attention, it is that of the sterols and steroid hormones. All animals and plants down to the lowly toadstools

and yeasts traffic in relatively large quantities of sterols. The human body contains some two ounces of cholesterol or bile sterol. This is a relatively inactive fatty substance whose chief function or raison d'etre is not fully understood. Much of the body's sterol is imprisoned with a life sentence in the human brain. Of the rest, about 20 gm. circulate each day in the bile. Each day at least 1 gm. of coprosterol is excreted; each day 1 to 2 gm. of plant sterols are ingested. The half period of circulation in the body is perhaps two days, the external ratio is about twenty-five days and the external half period about eighteen days, as short as the shortest vitamin half period. The significance of this to our argument you will see presently.

There is another group of steroids of the body of extremely potent character and these are present in very small amounts, at least fifty thousand times less than cholesterol. These are the hormones and many of them are near relatives of cholesterol. Cholesterol, you will remember, is the chief member derived from that four-ring chemical circus called the cyclopentano-phenanthrene nucleus and it has a long carbon chain tail on the "seventeenth" carbon atom. It is also supplied with two important methyl groups, one hydroxyl and a few double bonds. Remove the tail from cholesterol and add oxygen and we produce the male sex hormones. Then add a few double bonds and there result the female sex hormones. Add a keto group and—presto—there are the cortical hormones; go back to the start, and irradiate the cholesterol with ultraviolet light and one of the rings is broken, with the production of an active methylene group. The result is vitamin D, one of the most extraordinarily potent substances (that is to say active in the smallest quantity) known to the biochemist. Take off the methyls and the oxygen and fit double bonds to all the rings—then we have carcinogenic hydrocarbons.

However, does the body keep this chemistry straight with all these many highly specific and potent derivatives of cholesterol? How does the body avoid disaster? The answer I think is that it doesn't! I once wrote a story about an angel who asked to come down to earth. He was told that he would have to have a body where the heart must beat once each second and he must breathe

sixteen times each minute and if either stopped for an appreciable moment, he was more likely to be consigned to Hell than return to Heaven, and the angel decided the risk was too great. If he had heard about the steroid hormones, he would not even have asked his question!

I said the body does not keep its chemistry straight, nor does the kitchen gas stove. For every hundred molecules of carbon that are burnt under the family kettle, perhaps ninety-nine goes to harmless carbon dioxide but one goes to carbon monoxide, which is poisonous. It does not worry the cook and nobody dies from the gas stove unless he puts his head in the oven without lighting the gas. The reason is that the ordinary ventilation of the kitchen carries the carbon monoxide away so rapidly that it never accumulates harmfully. Occasionally, however, the burner gets out of kilter, and the window is closed too tightly, and then we say that the kitchen is stuffy. I suggest to you that the kitchen of the body becomes horribly stuffy toward the end of life and occasionally a cancer is the result. How does the healthy body keep its chemistry straight? First, it adjusts the burner to burn, giving the minimum toxic products. Then it arranges the ventilation to remove those that are inevitably formed and, finally it arranges to burn up in its own incinerator that which is not carried away by ventilation. I have spoken to you before about the incinerator system, the vitamin A, the quinones, and the essential fat peroxides. Let us consider now the ventilation system for the steroids.

Why the milligram and microgram quantities of the active steroids and the multigram quantity—amounting to a significant fraction of a kilogram—of cholesterol? What is the cholesterol doing, this apparently inactive substance. Biochemists talk vaguely of intermediary metabolism, of important functions in bile, and intestinal processes, and so forth, all of which may be true, but referring to our screwdriver hypothesis, I want to suggest one other important function of the cholesterol.

When a new radio element is formed in the cyclotron or the atom pile, how is it isolated? Manganese has been bombarded with neutrons and has produced radiostrontium. How are the few new molecules of strontium removed by the chemist? He puts them in

a solution with ordinary strontium and then precipitates them as the sulfate and all the new, rare isotopes come down with the common isotopes. I want to suggest to you that an important function of cholesterol may be to form a kind of isotope or homologue, a bandwagon onto which the rare and potent sterols can jump on their way in and out of the body or some special portion thereof.

The Polish ambassador comes to Washington to deliver an important message to the White House and to get there he takes a streetcar or a taxi. He could not create a taxi or a streetcar just when he wants it. It is because those vehicles are in daily operation for taking the ordinary man about his inconsequential affairs that they provide the ever-ready vehicle when the important occasion arrives and the maternity patient must rush to hospital or the ambassador must see the President. Pursuing this philosophy then, the hormone will be riding through the body in the steroid taxi. But, if after visiting the White House, the human steroid is metabolized into a polar bear, it is found that the polar bear cannot be accommodated in a taxi and, as a compromise, is shut in the White House Blue Room because nobody has bothered to think through what would happen if the animal were not conveyed away. And I suggest that after a shorter or longer time, the polar bear would cause cancer in the White House Blue Room.

So I ask the question rhetorically, is cancer or rather a major predisposition to cancer a symptom of aging caused by the inability to dispose of internal waste matter? Are not many cancers due to lack of internal cleanliness? I suggest to you that even if your present researches are of unchallenged importance and leading to worth-while destinations, as I am convinced they are, this consideration of in transitro chemistry must be made because it is conditioning all that you examine.

For instance, at your Endocrinology Panel this morning I heard a paper on the production of testicular tumors in mice by administration of sex hormone. Suppose the minimum dose for x per cent incidence were determined under ordinary conditions and then again under abnormally oxidative or reductive conditions or under buffered conditions. Suppose one of these conditions altered

the incidence of tumors—what a flutter there would be in the dovecotes of cancer research! And immediately we would have a clue and a prophylactic measure. You remember Dr. Tannenbaum's discovery that nitrophenols would reduce the incidence of mammary tumors in mice? An oxidative, scavenging phenomenon, I suggest. Or his work on diet restriction?—less waste to wash out. My hypothesis may be an over-simplification and to that extent misleading, but it does perhaps suggest a few new experiments.

Before I ask my final rhetorical question—Has the vacuum plumber a viewpoint of interest to the cancer chemist?—let me tell you a story about that up-to-the-minute chemical news agent, the isotope. When I was a student in London all too many years ago, the great Dr. Asten was perfecting his mass spectrograph, and after revealing the presence of many scores of natural isotopes, he published a book which we all eagerly bought. This little blue book had the simple inscription on the cover "Isotopes." I lived with this book; it was in my pocket in college, by my bed at night, and one day I took it to the dentist, read it in the waiting room, and afterward carried it into the awful presence. And the dentist, wishing to put me at my ease, picked up the book and said, with an air of nostalgia, "Ah, the Greek drama; it is many years since I read Isotto-pes." My friends, I do not know whether my speech tonight has concerned cancer or the Greek drama, but I must leave that to your merciful judgment. I wish you good fortune in your high endeavors.

FUNDAMENTAL SCIENTIFIC RESEARCH AND ITS APPLICATIONS

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When Dr. Cameron asked me to address the National Cancer Conference of the American Cancer Society and the National Cancer Institute I must confess that I was a little puzzled by the invitation just as I was flattered by it. What, I asked myself, could I possibly contribute to a meeting of men and women whose work is so highly specialized that the greater number of the subjects listed on the program seemed unintelligible to me

On further thought, however, although none of my diffidence disappeared, it seemed to me that a historian of science might have something of special interest to say to the biologists, physicists, chemists, and medical men gathered together to discuss problems related to cancer. As I conceive it, the history of science is far more than an antiquarian pursuit. If studied properly, it should lead to certain conclusions about the nature and progress of science itself, the way in which discoveries have been made in the past and will presumably continue to be made in the future, the conditions in the past that have proved inimical to scientific research and those that have been best for scientific progress. In other words, to the degree that the study of the history of science illuminates some of the problems of our own time, my remarks tonight may be especially appropriate to this occasion.

The primary job of the scientist, I take it, is today (as it has always been) to add to our understanding of the external world around us and of all the phenomena that occur in that external world. The ideal of pure scientific knowledge is an old one, going back in time at least to the early philosopher-scientist of ancient Greece. A curiosity about the "nature of things" has always been a chief motivation of scientific research; but it is clearly not the

only one that guides our pursuit today. Scientists, after all, live by their hearts as well as their heads; they cannot help being constantly drawn to the hope that their work will not only be at the service of "abstract truth," but that it may also help to make the world in which we live in some way better: to alleviate suffering, to raise the standard of human living, and to help in some measure, be it large or small, to give the rest of mankind better and easier lives. Indeed, one of the aspects of the whole scientific enterprise that marks it off abruptly from its fellow academic disciplines is: in proportion to the amount of pure knowledge gained, practical effects of all sorts quickly follow. Every worker in the field of botany, no matter what his job may be, knows that there is a strong possibility that his abstract researches may decrease the total sum of human hunger. The organic chemist, busy at making new syntheses, is always aware that he may soon have within his grasp a still more powerful insecticide or antibiotic.

I take it we are all gathered here because of our dual interest in advancing knowledge and in applying that knowledge where it may be needed. Let us look into the latter aspect, the way in which scientific knowledge is applied. My own interest in this subject derives from an initial impetus given me by Dr. William J. Robbins of the New York Botanical Garden, then Chairman of the National Science Fund (National Academy of Sciences). In company with Mr. Howland Sargent, Secretary of the Fund, Dr. Robbins asked me a number of years ago whether I would be willing to write a general book for the layman: showing just how the fundamental scientific research carried on to increase knowledge also yields practical dividends to the doctor, the investor, the manufacturer, the engineer, and the farmer. Supported by a research grant from Harvard University, I undertook a survey of this problem, which lasted for some five years. The results have been embodied in a book entitled *Science, Servant of Man,* published last autumn. During the course of this study I, and those who worked under my direction, turned to the published records of the past and the present. In addition, I personally spent a considerable amount of time interviewing men who themselves had either made important scientific discoveries or who had been con-

cerned in the task of applying scientific discoveries to useful ends. During this investigation, one clear and unmistakable observation was clear from the outset: during the last 300 years, the major part of the great practical advances in agriculture, in technology, in preventive and curative medicine, and in almost every aspect of human affairs, came from areas of scientific investigation that at the time had *no apparent connection with any practical issues* whatsoever, much less the ones to which they were finally applied. Even in cases in which a course of investigation was embarked upon because it seemed to relate to a practical need, it has often turned out that something equally practical or equally important was found, but not that which was originally sought. The best-known example, of course, is that of Hoffman's student, Perkin, who, about a century ago, attempted to synthesize quinine; he didn't succeed in making quinine, but he did discover the first coal-tar dye, or aniline dye, and the repercussions of that work in almost every area of science and human living are too well known to need further discussion.

The late Wilhelm Ostwald, the chemist who originated the famous series that bears his name, the "Ostwalds Klassiker," had the idea that if one made a serious enough study of the history of science, one could end up with a "super-science" of discovery. Therefore, he believed that as a result of historical study, the time would come when each job of research could be tailored to the particular end one had in view. Most historians of science today have less pretentious aims. They are fully satisfied if their investigations of the past simply helps us obtain a better understanding of the present. It is all too easy, looking at the record of science from the point of view of our own time and indulging in what Michael Polanyi has called "writing history backwards," to find obvious and immediate logical links between one step and the next, and between a discovery and its application. But, if, on the contrary, we look at any era of the past in terms of *its own perspectives* and point of view, we may find a closer kinship to our own time. Examined in this way, the "obvious" logical chain of the past, which so often seems lacking in the present, *turns out to have been equally nonexistent in the bygone days.* For clearly

the development of the sciences does *not* follow a simple logic. The second law of thermodynamics was found *before* the first, and examples of this sort could be multiplied endlessly.

If I may take a somewhat typical example of what I have in mind, I would point to a discovery held by men and women in the eighteenth century to be extremely important in their daily lives. I refer to the invention (or discovery) of the lightning rod. To most Americans, Benjamin Franklin is the very incarnation of the practical spirit, the man who wrote the mottoes for our savings banks, who founded our fire departments, our libraries, and so on. All that most of us know about his scientific work is that he flew a kite. We are often told that, typifying the *American*(!) spirit in science, he put his discoveries to work and invented the lightning rod. This homogeneous picture has, it must be admitted, a certain charm and a much greater appeal than the facts warrant. In the first place, those who claim that Franklin "directed his research toward practical ends" are guilty of a simple error in logic, which also betrays their ignorance of the nature of the scientific enterprise. Prior to the completion of Franklin's electrical research no one *knew* that the lightning discharge would prove to be an electrical phenomenon. How, then, could Franklin have possibly known that his research on Leyden jars, charged insulated conductors, the effects of grounding, the effect of shape on electrostatic conductors, and the like, would lead him to an explanation of the lightning discharge and a means of alleviating its pernicious effects. Franklin was as pure a scientist as any of us; he should be known not so much for his lightning rod and kite, as for the fact that he conceived the first adequate unitary theory of electrical action, of which we still use the words which he introduced such as "plus" and "minus," "positive" and "negative." But Franklin believed, as most of us do today, that most, if not all, knowledge of the external world learned through science will eventually find some application in the service of man. How delighted he must have been, therefore, when his scientific research led to the practical issue of the lightning rod. But that this was a wholly unexpected and unanticipated result is, I think, manifestly clear.

Let us turn to an example from more recent history. Much has been made in the last several years of the wonderful new selective weed-killers, of which "2, 4-D" has received the most popular éclat. I am certain that those of you who have homes or gardens will agree with me that the discovery of weed-killing plant hormones (or auxins) is a most useful and worth-while addition to man's skills. Some of you, I am sure, know the history of our knowledge of growth hormones, but others may not. It may come as a surprise to realize that this important and most practical subject had its origins in a series of experiments in which Charles Darwin tried to answer the question: What makes plants bend toward a source of light? From experiments in which matches were held for a short moment near oat seedlings growing in a dark closet, a group of plant physiologists over the years learned more and more of why this bending occurs and why certain other phenomena of plant growth occur in the way they do. Selective weed-killers are but one of the practical fruits of this research. Others include sprays for preventing preharvest drop of apples and for preserving certain fruits and vegetables during transportation, the production of new types of seedless vegetables and fruit, and so on. I think you will agree there is no obvious connection between these important end products and the initial question which Darwin asked. This topic shows us how fruitful for knowledge it is to ask *fundamental questions;* and also how the answers to such questions by our scientists provide revolutionary changes in our daily lives as well as a greater understanding of the world we live in.

During the course of my research project on the practical applications of fundamental research, I asked several of the scientists who had been engaged in the study of plant hormones in those earlier days (when the subject seemed to be *connected with growth,* but *not in any sense with the kind of practical applications which I have just described*) what their policy would have been had they been required to devote all of their attention to the weed-eradication problem. I was told that probably they would have given up their study of the bending of plants toward the light, the effect of cutting off the tip of a seedling and then gluing

it on a little to one side, and so on, but would rather have looked into the question of improving existing methods of weed killing. The result of this course of action clearly would have been that no fundamentally new method of weed killing would ever have been discovered.

Like the previous example of the lightning rode, the story of 2, 4-D shows how difficult it is to predict which field of research *today* will contain the germ of the practical application of *tomorrow*. It is clear, therefore, that the overall approach to any practical problem must be as wide and as general as possible, in order that no eventuality may be lost.

One of the problems that all scientists must face is the actual link between the discovery itself and its application. We may dismiss at the very outset the cases of supposed "time-lag" in which there is a late arrival of the need. I refer here to the fact that many branches of science prosecuted for the advancement of knowledge (because of that curiosity about the "nature of things" which motivates so much scientific research) contribute knowledge and skills that are at hand and ready to be applied when the need occurs. A striking example is provided in the work of those men who studied micropalaeontology, the study of microscopic-sized fossils. Prior to World War I, micropalaeontology must surely have seemed one of the most useless branches of science from the "practical" point of view. Yet when, in the twenties, there was suddenly an inordinate demand for oil, and when prospectors needed every tool and method that science could provide in order to make that search more efficient, the knowledge of the micropalaeontologist was available and put at the disposal of the oil men.

In other cases, however, there seems to be a true time-lag, not dictated by economic or self-interest, but by what nonscientists and would-be scientific "generals" wrongly, so I believe, attribute to lack of adequate "foresight," or of proper "planning." Those who are engaged in scientific research, or the successful administration and support of it, are often held up to criticism because a discovery of the utmost importance to mankind was not soon

enough brought to fruition and immediately put to use in the service of mankind.

As a last example, let me dwell on a little known aspect of a story from the recent history of science, which is well known to all of you: the development and introduction of the great antibiotic penicillin. You all know of the "happy accident" that occurred when, in 1928, Fleming noted the curious bacteriolytic effect of the *Penicillium* mold that had contaminated his dish. Furthermore, you all know that the successful completion of his observation awaited a decade until, on the eve of war, Florey and Chain and their co-workers produced the antibiotic that still bears the name given to it by Fleming: Penicillin. Why was it, you will surely wonder, that a decade passed before Florey, Chain, et al., completed the work begun ten years earlier by Fleming?

I do not profess to know all the answers. One apparent reason is the difficulty of the task itself in which Florey and his group were finally engaged. But, during those ten years, some very important events had occurred. For one thing, in 1928, the great golden dream of Paul Ehrlich of an era of chemotherapy had not been realized. Typical of the sentiments of the era is the statement made in 1929 that: "Ehrlich's ideal to effect the destruction of all the parasites of a disease by means of an internally administered chemical agent . . . has shown itself on biological grounds to be unattainable." Both chemotherapy, and a similar idea in the warfare against disease had led to dead ends: microbial antagonism—a field of investigation begun by the great Pasteur.

But between 1928 and 1938, several very important things had happened. In the first place, the German dye chemists who had plugged away at their work with an almost monotonous steadfastness had finally reaped the reward of the discovery of the sulfonamides. Immediately, there was a renewed interest in chemotherapy. Then, Dubois, working at the Rockefeller Institute, had discovered gramicidin, a naturally produced antibiotic. A new atmosphere was immediately discernible among all men of science and medicine. Attention was concentrated on antibiotics of all sorts, and it was now only natural that Florey and his group

should work on this program. They first tried their hand at pyocyanase, a secretion of the bacteria *Bacillus pyocyaneus;* when this proved unrewarding, they tried lysozyme, another of Fleming's discoveries. Then, lastly, they turned their efforts to penicillin with the well-known results.

I think this story shows that in science the recording of important individual facts, such as Fleming's observations on *Penicillium notatum,* do not alone, by and of themselves, constitute a complete discovery. Men's minds must be oriented toward a point of view which will accept and use this new fact and incorporate it into the life of science. Long before Fleming, many observers had noted the bacteriolytic effect of Penicillia. The earliest was found by H. Landsberg (of the Research and Development Board) to be that of John Tyndall of the Royal Institution who is known for his work disproving spontaneous generation. (See *Isis* 40:226, 1949.) He noted, in the 1870's, many examples in which the growth of bacteria was inhibited, and in which bacteria were actually destroyed, when his solution was contaminated by common mold, Penicillia. Tyndall's account was widely published and widely read, but it did not in any way affect the war against disease.

Such examples of isolated observations could be multiplied endlessly and to no avail. They show us that an observation of the greatest importance must fall upon receptive minds; and that in science the acceptance and use of a discovery requires that men's minds be in such a condition that individual facts gain significance and become used. I like to call this state of men's minds *the total scientific situation.* Every scientist lives and thinks in terms of it; and new discoveries, if they are to be more than isolated facts chiefly of interest to coming generations of historians, must fall upon the proper total scientific situation. All the more credit to Fleming, who preserved his penicillin-producing strain until men were ready to use it.

The importance of considering this example fully should be especially manifest to a group of scientists and clinicians interested in cancer. Every man and woman working on any aspect of this problem, like all other scientists, hopes that the results of experiment and study in the laboratory and hospital will not re-

main isolated facts, but will become part of the living structure of science. Yet the effect of any research work can be gauged only by the way in which it fits the total scientific situation. This raises the immediate question: how can men's minds be freed from their own times? I must confess that I do not believe there is an answer. Nevertheless, the very existence of this question must, in some degree, determine our conduct with regard to the scientific enterprise as a whole. We must make sure that any serious investigator will be free to continue with his work, even if to us, in the light of the total scientific situation of today, it does not seem that that particular direction is apt to be fruitful. After all, tomorrow, in a new total scientific situation, the apparently unfruitful work of yesterday may be of the most fundamental importance. Furthermore, as the case of the sulfonamides shows that apparently unfruitful work itself may be the determining factor in overthrowing the total scientific situation in which we are bound and in establishing the new horizon of tomorrow.

It is clear to every investigator, and to anyone who really understands the nature of science, that no one can ever tell which branch of today's science will be related to the important applications of tomorrow. In just the same way, furthermore, it is often most difficult to forecast at this moment which field of science itself will prove tomorrow to be the most exciting frontier for the explorations of "pure" science itself. As a result, we are led to the inescapable conclusion that all fields of scientific research must be encouraged. Perhaps a little financial pressure may be exerted to entice men into needed and neglected fields, although the recent *Fortune* poll has shown what all scientists have always known: that scientists are not activated in their choice of field by financial interest or reward.

The need of insuring that very field of work be prosecuted raises at once the major question of freedom for the scientific investigator, freedom to work on the job that he chooses for himself—dictated by his personal enthusiasm, emotional bent, his particular talents, and his training. How can we say that it would be better for a man to work on "Topic A" rather than on "Topic B"? Who knows what changes are occurring in the world of science at the

very moment that we make such a judgment? Will not tomorrow bring us a world of wholly new values, both in pure science and in the fields of scientific research that will appear to be related to this or to that practical problem? How can we predict the way in which the total scientific situation is changing at this very instant?

This leads me to the last point. Having mentioned freedom in science, I cannot escape the word "planning." This is a "loaded" word, especially because of its economic overtones. A man who believes in a complete "laissez-faire" in our economic life is, in this era, supposed to be a Conservative and Reactionary, or even a Tory, as contrasted to the "New Deal" Liberal who believes in some restriction or regulation of our economic life consistent with the greatest freedom or benefit for the greatest number of individuals in our society. By a false analogy, those who do not believe in scientific planning on the level of fundamental knowledge, or "pure" science, have become known in certain quarters as "tories in science," or "scientific reactionaries." If this be a crime then I must plead guilty of it. But I think that this confusion of economic and scientific ideas introduces a complete distortion of major values. Indeed, I believe it can be demonstrated that no matter whether we live in a completely laissez-faire economy, or in a liberal capitalism such as we have in the United States, or in a limited form of socialism such as we find in England, or even in a completely planned totalitarianism such as they have in Russia—no matter what form of economic life and aim we have, the best way to insure the improvement of agriculture, the development of technology, the advancement of preventative and curative medicine, is to give to those men and women engaged in scientific research the greatest freedom possible to make the discoveries that will be applied today and tomorrow. By freedom, I mean freedom from "general staffs," whether civilian or military (at least in peacetime), and also freedom for each scientist to choose his own field of work, to work at his self-chosen assignment in whatever way he feels he can do it best, and to know that the values and the nature of science are understood, so that his work will be supported even if it does not seem to be related imme-

diately to a practical problem. And lastly, I mean the freedom for every scientist to believe in whatever his heart and his conscience and his scientific experience teaches him without having his beliefs dictated by the politics of a state science.

Above all, if science is to continue to flourish, and if the practical applications we expect to get out of science are to be forthcoming, we must insure the support of science: scholarships and fellowships for the training of the men and women who will be engaged in scientific research, and freedom from interference by those who cannot see that the pursuit of knowledge is a wonderful thing itself. For only by encouraging to the widest extent possible the pursuit and increase of knowledge for its own sake, will the great discoveries be made.

Part Three

PANEL DISCUSSIONS

PANEL ON TUMORS OF THE REPRODUCTIVE TRACTS AND BREAST, INCLUDING ENDOCRINOLOGY

Moderator, C. P. RHOADS, M.D.
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Recorder, H. T. RANDALL, M. D.

CANCER OF THE BREAST

In Animals

Biological analysis has established that the etiology of breast cancer in mice involves three separate, but interacting, factors: (1) an inherited susceptibility, resulting from the action of at least two genes and possibly operating by way of a hormonal effect; (2) the milk factor, which is found in a number of the tissues of the animal bearing it, can be centrifugated, and in many respects is similar to a virus; and (3) a factor concerned with or resulting from the pregnancy of the animal, hence presumably also hormonal.

The evidence for the hormonal nature of inherited susceptibility is found, in part, in experiments on a strain of mice little susceptible to the induction of cancer by the milk agent. When castrated, animals of this strain show a pronounced adrenal hyperplasia. When males of this strain are crossed with females of a strain highly susceptible to the milk agent but in which there is a low incidence of cancer in the virgin females, the virgin females of the hybrids so produced have a high incidence of cancer. In this experiment, the inherited susceptibility due to the hormonal factor derives from the paternal parent and the milk-agent susceptibility from the maternal.

A similar cross with males of a strain not developing adrenal hyperplasia resulted in a low virginal incidence of carcinoma.

In a third set of experiments, males that on castration developed adrenal changes unlike this hyperplasia (changes that led eventually to neoplastic alterations) were used in crosses with females

susceptible to the milk factor. The virgin hybrids had a high incidence of cancer.

There seem to be differences in the potency of the milk agent in different strains, manifested by some difference in the incidence of

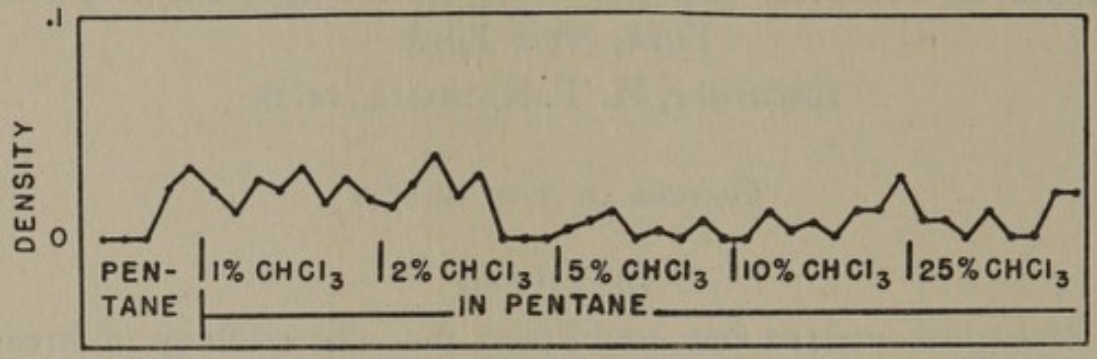


FIG. 1. Chromatogram on extract of feces from C3H mice with the milk factor.

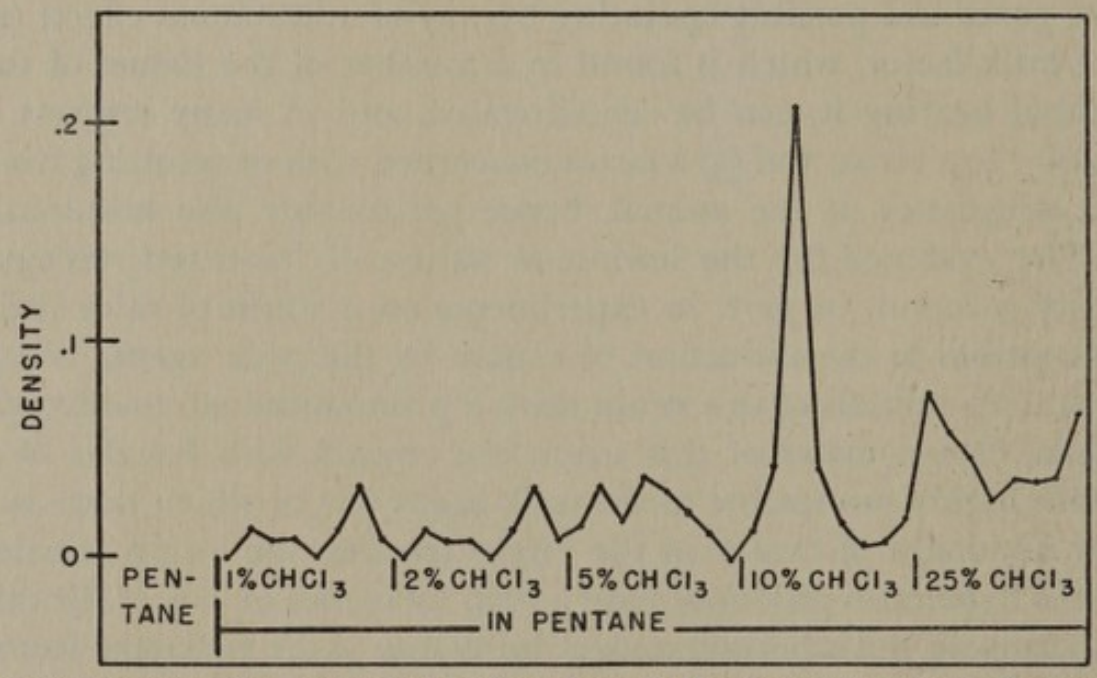


FIG. 2. Chromatogram on extract of feces from C3H mice without the milk factor.

carcinomas reported after foster nursing a test strain. Breast cancer may also occasionally occur in strains of mice in which the milk agent has not been demonstrated.

The third factor of well-established importance in the etiology of breast cancer is repeated pregnancies. In suitably selected animals that carry both the inherited hormonal factor and the milk

factor, but that have a low incidence of cancer in virgins, repeated pregnancies result in a high incidence of breast cancer.

Other experiments have indicated that stagnation of mammary secretions is an important factor in the breast cancer that occurs in force-bred animals. The studies here reported appear to eliminate secretion as a factor in the genesis of breast cancer under these experimental conditions.

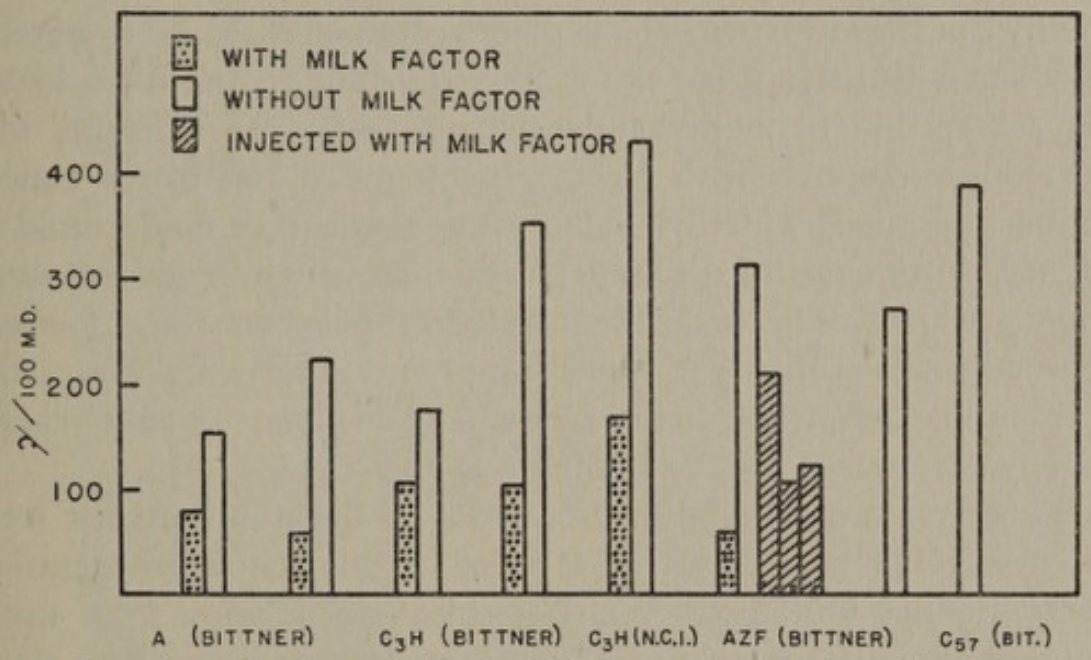


FIG. 3. Excretion of 17-ketosteroids by different strains of mice with and without the milk factor. Amounts expressed as γ/100 mouse days.

Differences have been found in the morphology of the ovaries in animals of two different high-incidence strains, one of which showed a low incidence of breast cancer in virgins. The F_1 generation of crosses between these strains failed to show the maternal ovarian characteristics, but the incidence of breast cancer in virgin females rose to that of the paternal strain. Similarly, ovarian-transplant studies showed that ovaries from either of the original strains could be transplanted into the F_1 hybrid after castration, but that high virginal incidence occurred in both transplants, and that the morphological differences characteristic of the maternal strain disappeared from the ovaries thus transplanted.

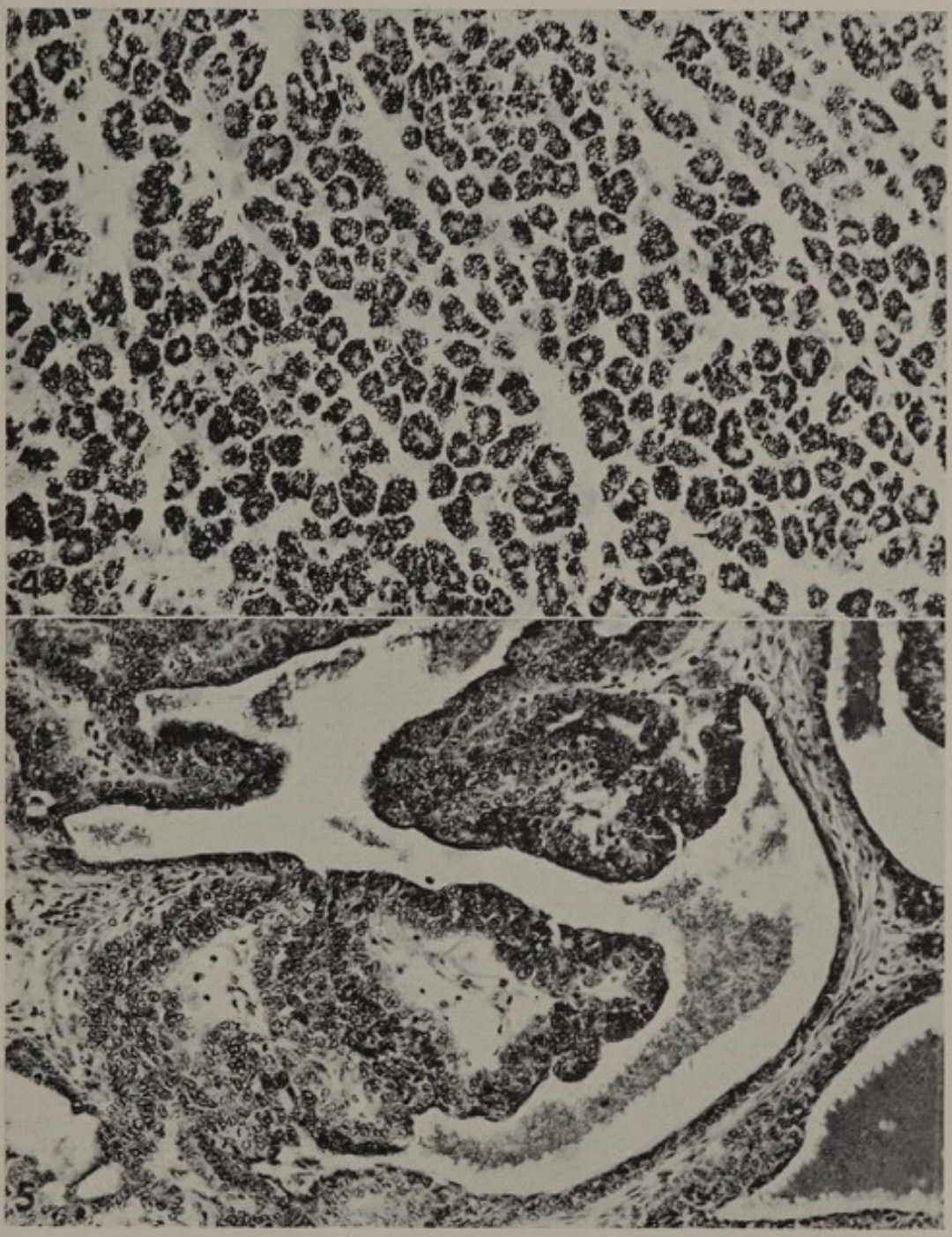
The importance of the genetic background in the incidence of breast carcinoma was emphasized, since inheritance controls sus-

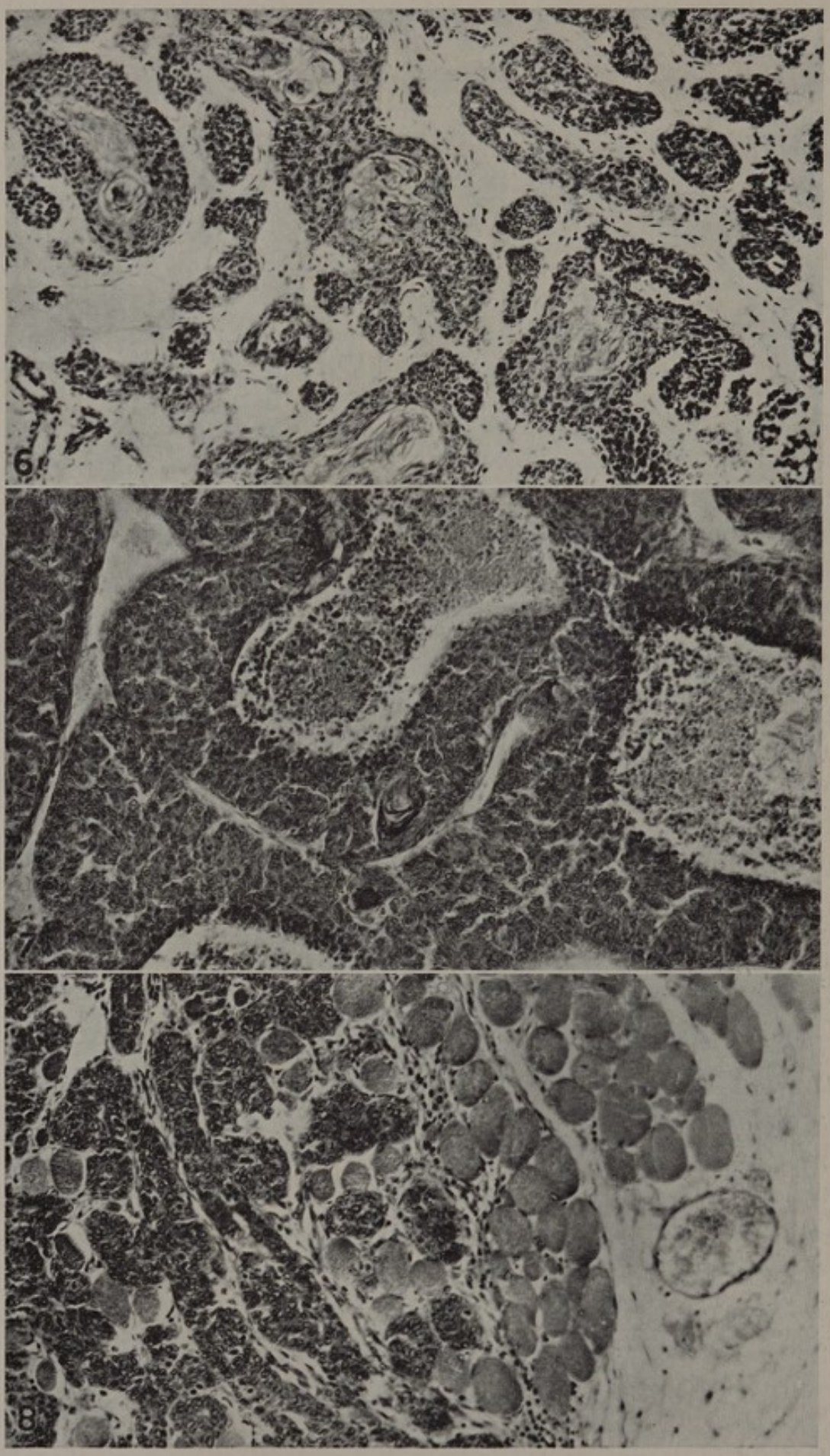
ceptibility to hormonal factors and perhaps, as well, the degree of estrogen level present for stimulation. As an example, two strains of rats were described, one of which shows an incidence of spontaneous breast carcinoma, while the other develops the disease only under the stimulation of supplemental estrogens. Estrogen therapy, on the other hand, is completely ineffectual in producing the disease in guinea pigs.

The susceptibility of strains of mice to the mice factor and to the hydrocarbon carcinogens provides another example of hereditary susceptibility: there are strains resistant to the milk factor yet susceptible to methylcholanthrene, while other strains will develop carcinomas with either. Incidentally, the breast cancer induced in mice by methylcholanthrene tends to be epidermoid in nature, histologically different from that which tends to occur spontaneously. The lack of correlation between the inherited characteristics of highly inbred strains of mice and human heredity was emphasized, there being inadequate evidence of any genetic pattern in human disease available at this time.

Three types of premalignant lesions of the mouse breast were discussed. The first consists of areas of intense acinar proliferation about a duct bud, further characterized by a more than usual amount of connective tissue. When these lesions are present, there is a high incidence of mammary carcinoma in the strain and carcinomas may sometimes be seen developing within the lesions themselves. Further evidence is suggested by the multiplicity of the lesion, which coincides with the multicentric origin so often seen in mouse breast carcinoma. It was emphasized, however, that not all these lesions become malignant. The second precancerous lesion was described as proliferation of alveoli within large areas of the breast, involving the structures of several ducts. The third consisted of areas of multiple proliferation of fine ducts extending radially from a focal point.

In order that these various precancerous lesions may appear in any quantity, all three major factors necessary for mammary carcinogenesis must apparently be present. If any one is absent, the precancerous lesions are essentially absent, and mammary cancer occurs only in a very low percentage of individuals.

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Once the precancerous lesion has developed in the mouse breast, it is irreversible. The administration of testosterone has no effect on it, nor does pregnancy affect its morphology. The precancerous lesions in estrinized male mice are the same as those in the female.

Experiments have been reported in which precancerous lesions were transplanted into hypophysectomized mice; a small number developed into full-blown neoplasms.

Certain lesions, not precancerous, were also described, including an inflammatory process characterized by lymphocytic infiltration with epithelial proliferation, often with associated squamous metaplasia, and a generalized subinvolution of the breast tissue following pregnancy. These lesions could not be associated with the development of spontaneous mammary cancer.

Study of the microscopic morphology of mouse breast carcinoma has shown that cytology is often a poor guide to malignancy, since adenomatous lesions of high differentiation are malignant in the mouse. Solid cords of tumor cells often grow out from these areas, and it is common for multiple cysts, often blood filled, to form. Frequently, there is papillary growth of carcinoma into the cyst spaces thus formed. Squamous metaplasia is fairly commonly seen and some breast lesions show participation of the mesenchymal elements in the form of sarcomatous areas. Occasionally in animals chronically exposed to roentgen rays, lesions may be predominantly sarcomatous in nature, although similar tumors have been rarely found in controls.

Contrasts were drawn between mouse and human breast carcinoma morphologically, mouse carcinomas being predominantly acinar, whereas human carcinomas are predominantly ductal in origin. Metastases in the mouse are primarily to the lung, and

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Figs. 4 to 8. Morphology of mammary tumors in mice. These photomicrographs illustrate the great variability which appears in different tumors and even in different areas in the same tumor.

FIG. 4. A small acinar structure, probably the most frequent and characteristic pattern.

FIG. 5. Papillary, intraceptic type of growth.

FIG. 6. Keratinized areas developing in center of nests of tumor cells.

FIG. 7. Cystic spaces formed by necrosis of tumor cells.

FIG. 8. Infiltrative growths into skeletal muscle at margin of a mammary tumor.

lymph-node metastases are rare, as compared to man. Also, there is little correlation in the mouse between the histology of the tumor and its tendency to metastasize. Bearing young increases the disease incidence in mice of certain strains and this is not true in man. Again, breast cancer in the mouse is of multicentric origin as compared with the more local origin usually presumed in man. Attention was also called to the profound anatomical difference between the breasts of mouse and man.

In dogs, fox terriers, Boston terriers, and cocker spaniels were reported to have a high incidence of breast cancer in comparison to the low frequency in Scotch terriers and German shepherds. Of 139 breast tumors studied in dogs, forty-seven were carcinomas; sixty-seven, mixed tumors; thirteen ductal papillomas, and eleven myoepitheliomas. The dogs ranged in age from 7 to 14 years. Infiltrating duct carcinoma was described as appearing in the dog and duct papillomas were considered to be precancerous lesions. Involvement of regional lymph nodes was described, as were pulmonary metastases. Carcinomas and mixed tumors were most common in the posterior glands. Cystic mastopathy was also described. One case of breast cancer in a male dog was mentioned, as were three intraductal papillomas.

Human Breast Cancer

The problem of the microscopic *diagnosis* of human breast cancer, with particular reference to the precancerous lesions, is essentially that of determining the point of irreversibility in the development of the chain of events leading to carcinoma—but it has been impossible to determine where this point lies. Even in breasts showing all stages of development of precancerous lesions and cancer, one cannot draw more than inferential conclusions with regard to the behavior of similar lesions in other breasts. The pathological diagnosis in carcinoma depends upon the observation of certain nuclear changes in cells in a characteristic relationship to each other. Yet, it was noted that identical pictures may be seen in certain virus diseases, in hepatitis, cervicitis secondary to trichomonas vaginitis, and most markedly in the human placenta. The analogy between placental growth and neoplastic growth was emphasized.

Reference was made to the contention that the functional behavior of cells transplanted under special conditions can be used as an index of their neoplastic properties, and it was stated that the assumptions upon which this claim is based are inadequate. The need for more adequate cytological criteria for the diagnosis of cancer was emphasized with particular reference to the development of cytochemical methods that would allow the differentiation of normal from neoplastic cells on absolute grounds.

With regard to diagnosis, the point was stressed that the only safe way to proceed with a lump in the breast is to take a biopsy and refer it to a good pathologist. The competence of pathologists is of extreme importance in the accurate classification of disease, with particular reference to axillary metastases.

Incidence. The fallaciousness of the close application of animal data to human disease was elaborated upon. Certain substrains of the human race have less breast carcinoma than others, notably the Japanese, but the genetic pattern in the human race is, as yet, poorly defined. It is probable, however, that women with a familial history of breast carcinoma have a higher incidence of the disease than have those without such a history. It was concluded that it would be premature to extend the idea of an extrachromosomal factor to human cancer of the breast.

There is certainly a hormonal influence in the human disease, shown by a female-to-male ratio of 99 : 1. However, women who have borne children have a statistically lower incidence of breast cancer than those who have not (with the possible exception of those with five or more pregnancies), the ratio being stated as 1.6-2 : 1. Breast carcinoma in nulliparous women may be in part due to endocrine disorders that affect both their reproductive ability and their incidence of breast disease. The tendency of cancer to occur in the opposite breast was described as about ten times that of the normal incidence of cancer. Human breast cancer may be multicentric in origin.

Attention was called to a reported experiment in which unilateral ligation of mammary ducts resulted in an increased incidence of cancer in mice, presumably due to retained secretion.

There has been no conclusive evidence that the use of estrogens increased the incidence of carcinoma of the breast in women.

However, the recent reports of breast cancer in males treated with estrogens for prostatic carcinoma make re-evaluation of this point necessary.

Trauma probably plays no role in producing breast cancer.

The similarity of surgical results in the *treatment* of carcinoma of the breast among the various major clinics recently reporting was noted and minor differences were interpreted as probably the result of variations in the criteria of operability. Exception was taken to the practice of placing certain types of carcinoma of the breast in the categorically inoperable group in view of a recent report of a small percentage of salvage in such cases. It was suggested that a somewhat more liberal interpretation of operability might result in the saving of a few cases. Thus, 75 per cent of patients seen at the Massachusetts General Hospital were considered operable.

A series was presented from Memorial Hospital correlating the size of the mass in the breast at the time of operation with five-year-cure rates. It was shown that in masses less than 1 cm. in diameter the cure rate is 92.9 per cent for five years, the rate decreasing as the size of the mass increases. It was stated that it is the impression that axillary involvement was less in mass if not in percentage as the result of earlier treatment.

It was not believed that the results of more radical mastectomy as yet justified the prolongation of operative time entailed. A recent series in which the extent of the operation was increased, including supraclavicular dissection particularly for carcinomas of the inner quadrants of the breast, is in an early stage and no statistics are yet available.

A study was referred to in which the percentages of involved axillae were compared in patients whose disease recurred in the other breast. At the original operation, 63 per cent showed involved axillary nodes, but when a second operation was done for disease in the opposite breast, only one third showed axillary involvement. Since the patients were examined every three months after the first operation, the early diagnosis of disease in the second breast was thought to be the reason for the lower incidence of disease in the axilla.

Pre- and postoperative radiation was found to have failed to give any improvement in most cases when cure rates were analyzed.

The mutilation that results from trying to cure breast cancer by irradiation is quite as serious, if not more so, than that which follows surgery. Figures were presented showing a 26 per cent five-year cure in 126 cases treated by radiotherapy, which is about one half of the present cure rates with radical mastectomy. When axillary metastases are present, the results were markedly inferior to the results from surgery.

Improvement in the results of therapy can be accomplished primarily by early diagnosis and operation. Great stress was laid on this point together with the need for continuing education of both the public and the medical profession itself.

Among factors that cause tardy appearance of patients for treatment are those of fear, ignorance, and temporizing advice given by local doctors. This last point is one of the important points of attack for cancer education. The point was made that self-examination was probably good in younger women but not as effective in the older age group. The importance of cancer detection clinics and the increasing public interest in them was stressed.

Carcinoma of the Cervix

Spontaneous carcinoma of the cervix in mice is rare, in general, and does not appear to be induced by the carcinogens. In one strain, however, 50 per cent of the animals will develop carcinoma of the cervix when estrogens are given, small quantities being more effective probably because of longer survival periods. It was also reported that 50 per cent of 500-day survivors with simultaneous estrogen and androgen treatment developed disease, possibly owing to the suppression of the development of leukemias associated with estrogen therapy. The lesions are predominantly epidermoid. In the PM stock, there is a spontaneous incidence of carcinoma of the cervix that is increased with estrogen treatment together with a suppression of the incidence of spontaneous fibromyomata.

Cervical carcinoma has been found to have a higher *incidence* in lower income groups, perhaps due to a combination of diet, the

trauma of multiple births and poor obstetrical care, and possibly to some racial influence. Genital hygiene may be a possible etiological factor: the Jewish race shows a remarkably low incidence of cervical carcinoma, the Negro the highest.

In the routine examination of 1319 women, all more than 30 years of age, three carcinomas were found; one more was detected during the ten-year period that 732 of these were under continued observation. In a comparative series, six cervical carcinomas were found in 1000 single women; sixteen, in 1000 married nulliparous women; thirty-seven, in 1000 parous women; and forty-two, in 1000 Negro women. Reference was made to the high incidence of carcinoma of the cervix in India, with more cases among the Hindu than among the Moslem women. Circumcision is practiced by the Moslems.

There are three *primary presenting types* of carcinoma of the cervix: the papillary everting lesions with local spread; the ulcerating infiltrating type that spreads by contiguity but is invasive from its onset; and the endocervical type that often shows extensive local invasion. Induration of the parametrial structures is not an accurate aid in the evaluation of invasion, owing to the difficulty of differentiating between neoplastic invasion and pelvic inflammatory disease.

Four grades of carcinoma of the cervix were discussed with a suggestion that re-evaluation and further subdivision seemed in order. Grade-I carcinoma consists of tumor confined to the cervix. Size is not supposed to be of importance, and yet 17 per cent of lymph-node metastases were reported in a series of Grade-I carcinomas. Grade-II consists of carcinoma that may invade the uterus, parametrium, or vagina, and yet patients with this degree of involvement may die of the disease; 28 per cent of regional-node involvement was reported in this group. Grade-III includes infiltration of the pelvic wall to the introitus or isolated metastases on the pelvic wall; 40 per cent regional-node involvement was found in this group of cases, and yet a considerable portion of them may be operable. Grade-IV comprises cases with tumor involving the bladder or rectum, and those cases presenting distant metastases. With the exception of those with distant metastases,

some Grade-IV cases are curable by surgery. In a series of Wertheim operations, 40 per cent of the cases were shown to have no regional-node metastases. A similar autopsy series was also reported. Recurrence in the vaginal stump is rare if an adequate cuff is excised. Some cases with pulmonary metastases, without regional-node involvement, have been observed.

It was reported that at the Presbyterian Hospital in New York, Grade-I tumors are now being subdivided.

Carcinoma in situ was mentioned as being of very low-grade malignancy with invasion in only a small percentage of late cases.

Some cases of carcinoma of the cervix are radioresistant for reasons that are not clearly understood. It has been suggested that the resistant cells are more independent.

The Papanicolaou technique for the examination of vaginal smears was stated to be of proved value: positive tests will be obtained in 90 per cent of all cases with carcinoma and in 96 per cent of all with desquamated cells. False positives are steadily reduced by experience, there having been none in two years in the Vincent Laboratory. The diagnostic specificity of this test is not so good in carcinoma of the endometrium.

The technique is not a substitute for, but a complement to, biopsy. Thus, in a series of 181 cases, biopsy proved accurate in 90 per cent and smear in 91 per cent, but a combination of the two methods yielded 98.5 per cent correct diagnoses. Large tumors can give a negative outcome because of necrosis with destruction of cell morphology. The method has the advantage of giving a general sample of cells, while biopsy sites are difficult to choose in very early lesions. A series of thirty early, unsuspected lesions were reported being detected by this technique.

In *treatment,* the suggestion was put forth that the unhealthy cervix should not be neglected, that cervical erosions should be excised and adequate pathological examinations made, and that subsequent cauterization should be practiced.

Studies on the comparative merits of methods of treating cervical cancer were described. In one series, using what amounted to the local application of radium alone, 22 per cent five-year cures were obtained; 28 per cent were obtained when "massive" doses

of external irradiation were used in addition. In a third series, external irradiation was given by the divided-dose technique; cure rates were 34 per cent.

In a later study, two groups of patients from the same institution, composed of patients with diseases as nearly similar as possible, were selected. The comparative merits of irradiation by radium needles and by the cone technique were established. The complications were serious from the use of needles. It was suggested that the needles employed gave too high an intensity of irradiation, and a later study was referred to in which needles containing less radioactive material were employed, with a lessening of complications. The five-year-survival rates were better in the series treated by the cone technique than in the one treated by needles. A final series of patients was selected in which the result of Wertheim hysterectomies was compared with that of irradiation by the divided dose and the cone technique. About forty-five patients were in each group. Not more than three years have elapsed since the institution of this experiment, but the suggestion is at hand that a somewhat greater survival rate may be found in those patients treated by irradiation. About one third of these patients had Grade-I carcinoma.

Cancer of the Testis

In Animals

In fowl, teratomas have been produced by the injection of zinc chloride. The direct injection of carcinogenic hydrocarbons has produced fibrosarcomas of the testis in mice; and several laboratories have produced interstitial-cell tumors by the injection of estrogen. A marked hypertrophy of the male reproductive tract ensues on estrogen treatment, characterized by overdevelopment of the seminal vesicles and prostate. Of fifty-six animals surviving eight months of estrogen treatment, thirty-nine testicular tumors developed and twenty-six of these had lymph-node metastases; thirty-five of the thirty-nine manifested androgenic overactivity as shown by overstimulation of the accessory reproductive-tract struc-

tures. The testicular tumors themselves were of the Leydig-cell type (interstitial-cell).

It was postulated that the following sequence of events, observed microscopically, led to the development of the tumors: (1) an initial hypertrophy of the Leydig cells, with the occurrence of pigment in other cells present in the interstitial area; (2) a marked increase in the number of pigmented cells, together with disintegration of the Leydig cells, from which it was concluded that the pigment cells were probably macrophages; (3) virtual disappearance of the Leydig cells, together with the appearance of small areas of active regeneration; (4) the rapid growth of regenerating Leydig cells, despite continued estrogen therapy, with the development of larger than normal Leydig cells, many with acidophilic cytoplasm; (5) the reduction of the number of large cells; (6) the occurrence of nodules of small Leydig cells, often to the exclusion of all other types. This last phase was followed by the development of frank tumors.

Spontaneous tumors of this type occur in dogs, horses, and rats, and probably in man, and parallel morphological changes have been observed in old dogs. The point was made that if a similar histogenesis of interstitial-cell testicular tumors could be shown for mouse and man and if the details of the action of the endocrine that stimulates the formation of this tumor in the mouse were known, the mechanism of tumor development in man might be postulated by analogy. Possible explanations of the phenomena include the theory that estrogen may act directly on the testes as do the carcinogenic hydrocarbons and that there is interference with the normal differentiation of Leydig cells through estrogen influence on the pituitary. With regard to the first postulate, it was noted that carcinogenic substances produce a different type of tumor than is found with estrogens. Experimental evidence was introduced to show that animals treated with daily injections of pregnant mare serum for one year developed nodular masses of Leydig cells within the testes but no true tumors. Further evidence showed that testicular transplants from 1-day-old mice to the neck of sister litter mates resulted in the formation of Leydig-cell

nodules in the transplanted testes as well as in profound interference with the endocrine balance of the host's reproductive system, as shown by morphological evidence. It was postulated that there are multiple stimuli to the development of the Leydig cells and that estrogens interfere with the function of some of these. The suggestion was made that hypophysectomized animals might tolerate simultaneous gonadotropic and estrogen administration and so could be used to evaluate further the role of the pituitary. The question was raised as to how a biological effect that requires androgen can exist and, indeed, be intensified by the presence of administered estrogen.

The various methods of distinguishing follicle-stimulating hormone from chorionic gonadotropic hormone were discussed, including the use of hypophysectomized female rats, doves, the *Xenopus* frog, and the bill color of the English sparrow. Experiments were described using 21-day-old female mice, injected five times with various amounts of test urine, then killed from 96 to 100 hours later, and the ovaries examined. Follicle-stimulating hormones produced numerous ripe follicles in the ovaries of these animals, while pregnancy urine produced several corpora lutea and only a few follicles. Again, 147 cases of testicular tumor were tested, sixty-eight of which had known tumor at the time of the test. Of these, forty showed evidences of chorionic-hormone excretion and all but two are dead. About one fourth of those that did not excrete chorionic hormone died of their tumors. Thus, the excretion of chorionic gonadotropic hormone in cases of testicular tumor is a very grave prognostic sign. There was little correlation between the morphology of the tumor and the type of hormone excreted. There did, however, seem to be a tendency for those with seminomas to excrete follicle-stimulating hormone, while those with embryonal carcinomas or chorioepitheliomas excreted chorionic gonadotropin more commonly. There are two general types of testicular tumors, the seminoma and the teratoma, which contains chorionic cells. A sharp distinction cannot be drawn, however, because metastases from seminomas may contain chorionic elements, and both types probably arise from pluripotential cells.

Probably chorionic-gonadotropin excretion occurs when chori-

onic elements are present in the tumor. These elements are radioresistant; therefore, the prognosis is very poor. The presence of the follicle-stimulating hormone, however, is no index to whether or not tumor is present. Evidence was advanced that this hormone may be excreted after the tumor has been completely extirpated. This suggests that there may be an endocrine origin in tumors characterized by the excretion of follicle-stimulating hormone. Further evidence was presented to support this contention in that, in the Army series, 11 per cent of testicular tumors occurred in cryptorchid individuals, while in the population, the incidence of cryptorchidism is about 0.4 per cent. Six of twenty-seven cases observed in cryptorchids occurred in the descended testis. The inference was drawn that the same endocrine imbalance that causes cryptorchidism may cause testicular tumors. Eleven per cent occurred in pseudohermaphrodites, the highest incidence being in the 35- to 39-year age group. Tumors of similar types to those of the testes also occur often in hermaphroditic women. Trauma probably does not play a role, for of 845 tumors recorded in cryptorchids, 345 were in an intra-abdominal testis not subject to trauma. In this group of testicular tumors, the urinary excretion of androgen is low while the estrogen rate is normal to high.

Testicles from day-old rats were transplanted into the spleen in a series of fifty castrate male and fifty castrate female rats, with controls. Twelve tumors, mostly Leydig-cell, developed in the males, sixteen in the females; none appeared in the controls.

Testicular Tumors in Man

The Army Institute series of testicular tumors was reported: 1208 tumors from males, almost wholly of the draft-age group. Ninety-seven per cent of these tumors were teratoids, with a few interstitial-cell tumors, a rare metastatic tumor, and occasional angiomas, fibromas, etc.

The teratoid type was subdivided into four basic groups: teratoma, seminoma, embryonal carcinoma, and choriocarcinoma. The survival rate was 95 per cent in those with teratomas that showed smooth muscle surrounding an epithelial-lined cyst or connective tissue with central squamous epithelium. Of those dying, death

was attributed either to a cancerous area missed on pathological examination or perhaps to the fact that all testicular tumors may go through a phase of malignancy and metastasize before becoming differentiated into their ultimate types. The seminomas are gray, granular, circumscribed, but not encapsulated, masses that consist of sheets of cells separated by connective-tissue trabeculi. Most of the cells show a clear cytoplasm. Infiltration of lymphocytes and plasma cells was reported as common. The five-year cure rate was reported as 70 per cent by a combination of roentgen-ray therapy and surgery.

The seminoma is almost exclusively a tumor of the testis. Metastases are usually of the embryonal-cell–carcinoma type. Trophoblastic elements are occasionally encountered.

Embryonal carcinoma is a lobulated tumor with dense connective-tissue septa and consists of epithelial-like cells with numerous foci of hemorrhage and necrosis. The cells are anaplastic with prominent nuclei and basophilic cytoplasm. Cell walls are indistinct. Some cells may be multinucleated and resemble syncytial cells. It was felt that this might show the origin of these tumors from cytotrophoblasts. Some areas of coalescent vacuoles and adjacent cells also suggest this origin. The five-year cure, with radical surgery and radiotherapy, is from 20 per cent to 35 per cent.

The choriocarcinoma is characterized by cytotrophoblasts and syncytial trophoblasts. It metastasizes early and is radioresistant. The five-year follow-up is zero. Overlapping of types is seen from a series of 237 tumors on which 368 diagnoses were made. Of these, 108 were teratoma; 100 seminoma; 144 embryonal carcinoma and sixteen chorioepithelioma. The seminoma is most nearly a pure type, with 71 per cent of seminomas showing only one type of cells, while in general 18 per cent of testicular tumors are of one cell type only.

A rounded, nodular formation in some testicular tumors, characterized by a cystic structure of flattened and cuboidal cells accompanied by syncytial trophoblasts and surrounded by a loose mesenchyme, closely resembles the early stages of human-embryo development.

The theory was advanced that all testicular tumors may arise

from the basic sex cells, the seminoma arising directly, the teratoma from the somatic elements, and the embryonal carcinoma and chorioepithelioma from the trophoblastic elements of a parthenogenetic embryo. The tumors arising from the trophoblastic elements are the ones that excrete chorionic gonadotropic hormone.

Cancer of the Prostate

Autopsy statistics on men more than 50 years old show an *incidence* of 14 to 29 per cent of carcinomas of the prostate. Of fifty prostates serially sectioned, 48 per cent showed occult carcinoma. However, there are about 8000 deaths reported annually from this disease so that most of the occult carcinomas never assume clinical significance. The incidence of the disease rises progressively with age, but the factors which activate it are unknown.

It was stated that there are *three types* of prostatic tumors; sarcoma, half of which occur in children; epidermoid carcinoma, probably arising from the prostatic utricle, which is very rare; and the columnar-cell carcinoma, which is the usual tumor. Of the columnar-cell carcinomas, three kinds are mentioned. The latent, which is of no significance to the patient; the occult, in which metastases may be the first symptom and in which the tumor is small; and the clinical tumor of the gland. Of prostatic cancer, 75 per cent to 90 per cent arise in the posterior lobe. Latent tumors are very rare before 40 years of age, increase progressively, and are often of multicentric origin. Prostatic carcinoma bears little relationship to nodular hyperplasia, only 2 to 3 per cent of latent carcinomas being seen in hyperplastic nodules. The rest arise outside the areas of nodular hyperplasia.

Diagnosis. From a diagnostic standpoint, palpation is still the most accurate procedure. Prostatic smears are being investigated, but considerable difficulty is experienced in attempting to differentiate Grade-I tumors from normal secretions. In one series, 3 per cent positives were picked up in a hundred clinically benign prostates. In a series of sixteen patients, a 30 per cent error was encountered in establishing the presence of prostate cancer by smears of prostatic secretion. It was brought out that 90 per cent

of carcinomas of the prostate are inoperable by radical prostatectomy when first seen, and early metastases occur so commonly that more radical surgery probably offers but a small increase in cure.

Treatment. More radical surgery for prostatic carcinoma was urged, the route of spread of the disease being described as to the posterior capsule and then upward to the seminal vesicles. Involvement of the seminal vesicles is not a contraindication to operation. Many patients with prostatic carcinoma die of secondary urinary-tract obstruction and infection and a series of 139 autopsy cases showed that 22 per cent had no evident metastases on routine autopsy. The figure of 90 per cent inoperability could be reduced by the inclusion of a cystectomy with the operative procedure. The para-aortic lymph nodes are the most common site of metastasis, but the iliac nodes are second, and one report suggested frequent involvement of the obturator nodes in a small series of cases.

Early treatment of carcinoma of the prostate either by castration or by administration of small doses of estrogens has given fair results with weeks to years of remission. Most prostatic carcinomas eventually relapse under estrogen therapy, and when this occurs, subsequent castration is of no effect. The necessity of androgen for the growth of prostatic carcinoma has not been proved in all cases. In most cases, however, the disease gets rapidly worse when testosterone is administered. The adrenal gland may also produce androgenic substances. Hence, adrenalectomy has been tried in otherwise hopeless cases, on the hypothesis that the adrenal had taken over testicular function. Survivals have been of very short duration.

On the assumption that pituitary stimulation of the adrenal would affect tumor growth, roentgen-ray therapy to the pituitary has been given in doses of 1500 to 2000 r with equivocal effect. Local roentgen-ray, urethane, or HN_2 treatment is of doubtful value. Administration of progesterone provides another method of inhibiting pituitary activity. It was administered to twelve patients who had relapsed following castration and estrogen therapy, and incomplete evidence of improvement was obtained. In two cases, the improvement was striking but of very short duration. The

criteria of improvement were a decrease in acid-phosphatase level and subjective relief.

A case was reported in which pain recurred, within five hours of the administration of testosterone, in a patient carried on estrogen therapy. Estrogen, it was suggested, may make the tumor less malignant for a time, but the tumor subsequently breaks through the restraint thus imposed and relapses occur. These tumors tend to become progressively more malignant. In one case quoted, repeated biopsies of lymph nodes were made while the patient was under stilbestrol therapy. They showed generalized necrosis of the metastatic tumor, but some visible cells appeared in all of the twelve biopsies. It was also suggested that, perhaps, some cells never are responsive to estrogen inhibition and continue to grow, or that there is an upset in the hormone balance of the tumor to which it later adjusts itself with subsequent growth. It was noted that in mouse leukemia treated with antifolic compounds, the disease is held in check for four or five weeks and then escapes, but these cells, when transplanted to another mouse, are just as sensitive as they were originally.

Some differences between the effects of castration and estrogen were brought out, pain relief being much more rapid with castration; also, there were differences in the hormone output in urine and some difference in the phosphatase response. With castration, there is a temporary drop in 17-ketosteroid excretion, followed by a rise. There is a definite drop in the estrogen excretion and a rise in follicle-stimulating hormone. With estrogen administration, follicle-stimulating hormone is not materially changed, estrogen levels rise, and 17-ketosteroids drop. Another observer stated that 17-ketosteroid excretion was variable following castration and may rise, but that in castrates with estrogen, there was usually no rise. The thought was expressed that there is a need for better methods of quantitative assay of the various steroid-excretion products.

Reporting in the Literature on Clinical Cancer of the Reproductive Organs

Daland reported on the recommendations of the Panel on Recording of Clinical Cancer. The question of the significance of

two- and three-year survival statistics was discussed, particularly as it pertained to the older age group in which mortality from intercurrent disease makes five-year follow-ups difficult. It was decided that in skin, lip, and ear cancer and possibly in some other neoplasms, where the natural history of untreated disease gives a very short life span, two- and three-year statistics may be valid. The natural history of various forms of untreated cancer is available from the Committee. Standardized forms are available for the use of those who wish them.

Hormones in Growth and Development

Gonadal secretion of hormones in the embryo is unproved except in the freemartin.

In opossums, the growth of the accessory ducts was equally stimulated when treated directly with either androgens or estrogens, and there was no interference with the accessory systems of the opposite sex. Removing ovaries from 20-day-old opossums had no effect on the growth of the accessory female ducts; nor did castration, on the development of the prostate. The point at which tissues become responsive to gonadal hormone is variable.

In some placental animals, hormones have no effect up to the time of birth. The statement was made that absence of vitamin A in the diet in pregnancy is reflected in numerous defects of the genital tracts of experimental animals. It was also brought out that mice, castrated at birth, continue to develop and differentiate breast tissue, owing to the assumption of hormone production by the adrenal cortex.

The development of the decidua in the uterus was described as resulting from the combined effect of estrogen followed by progesterone, and that a persistence of a high level of estrogen prevents the development of the decidua. High estrogen levels prevent the transportation of fertilized ova by the fallopian tube and prevent implantation by inhibiting the formation of the secretory phase of the endometrium that is a necessary precursor. Progesterone is essential not only to implantation but to the growth of the embryo in rabbits. In them, oophorectomy on or before the twenty-eighth or twenty-ninth day of pregnancy is followed by immediate de-

livery; 2 mg. progesterone daily will prolong gestation until the fetus is at least twice normal size. In man, progesterone is essential to implantation, and small, 10 to 25 mg., daily doses are sufficient to produce a secretory phase of the endometrium. The administration of estrogens kills the fetuses in rabbits, but they are retained by the uterus until the estrogen is stopped. In pigs, the corpus luteum ceases to contain high levels of progesterone in the period immediately before delivery. Thus, the process of delivery appears to be an endocrine crisis. In man, oophorectomy after the third month of pregnancy does not interfere with pregnancy nor with the high level of steroid excretion. The placenta produces progesterone, which is excreted as pregnandiol, with rapidly rising urinary levels starting at about the hundredth day of pregnancy. This level does not fall sharply immediately prior to delivery. Certain changes in the ratio of conjugated and free estrogens are observed immediately preceding delivery.

In chicks, an increase of fifty times in genital-tract weight is observed in six days of estrogen therapy. It has been shown that folic acid must be present in the diet to permit this growth. When antifolic substances were administered with estrogens, there was a quantitative suppression of this growth rate, which, however, never reached the normal baseline. A quantitative relationship of one part of aminopterin to two parts of folic acid by weight was exhibited. It was stated that thiamine can substitute for folic acid in some bacterial growth. Hence, folic acid may be involved in the synthesis of purines and paramidines. 2,6-Diaminopurine suppresses the genital growth response to estrogens in chicks, thus showing antifolic activity. The addition of adenine prevents this effect.

Hormone-Induced Tumors

A great variety of tumors can be induced by hormones. In some cases, hormonal increments may be intrinsic, as in a strain of mice with 5 per cent virginal breast carcinoma, the incidence of which rose to 25 per cent with sterile breeding and pseudopregnancy. In male mice, castrated and with the mammary-tumor agent, carcinoma of the breast arises in some strains but not in others. Even

with hypertrophy of the adrenal cortex and morphological mammary development, tumors do not occur unless a basophilic pituitary adenoma is also present, and there is a genetic pattern for the production of this pituitary lesion under these circumstances. Without the tumor, alveolar growth does not occur although duct hypertrophy does. Thus, four factors are necessary for carcinoma of the breast in male mice of some strains: the genetic type, hormonal activity (adrenal), mammary-tumor agent, and a pituitary adenoma. The development of these lesions may be prevented by treatment with strongly active estrogens or androgens but not by progesterone.

Endocrine Alteration and Excretion Studies in Breast Cancer

The question of the benefits of castration in carcinoma of the human breast was then discussed. It was suggested that surgical castration might be somewhat more effective, since estrogen activity exists in 30 per cent of patients castrated by roentgen rays. A series of fifty cases castrated largely by radiotherapy was compared with two groups of controls (one, premenopausal, the other more than 60 years of age). Premenopausal women, without axillary-node metastases, have such a good prognosis that castration was considered unjustified. Carcinoma of the breast appeared more malignant and is usually more advanced when first seen in younger age groups, but in comparable groups, premenopausal patients with artificial menopause behaved in exactly the same fashion as did the two control groups. It was brought out that, in mice, oophorectomy at 9 months of age had no effect on the ultimate incidence of the disease, but when the animals were castrated at progressively earlier ages, the reduction in incidence became apparent. Mice castrated at 18 days of age had no breast carcinoma. It was observed that the adrenal cortex takes over ovarian function under many circumstances.

Carcinoma in the human male breast is usually far advanced when first seen. One case, treated with testosterone, showed rapid expansion of the lesion. In one series, castration was done in a total of thirteen cases with long periods of regression and healing in some. Frank failures were reported in a man of 30 years and

in a case of inflammatory carcinoma. Of the thirteen cases treated, six are dead and seven alive for periods up to five years. Small doses of estrogen had no effect in several of these cases.

In women, initial attempts with 25 mg. of testosterone, three times a week, were reported; half the patients felt better and had relief of pain. High serum-calcium levels were encountered and considered a dangerous reaction. In another series given 100 mg. testosterone three times a week for eight weeks, there was roentgen-ray evidence of temporary resolution of body metastases in many cases and soft-part regression in some. It was observed that spontaneous hypercalcemia is not uncommon in breast carcinoma, it being found in 10 per cent of cases with osteolytic metastases. Four cases occurring during testosterone administration were recorded, all in patients in bed and all in patients with evidence of badly damaged kidneys. It was suggested that extensive renal damage was a contraindication of the use of testosterone.

Of patients with bony metastases given testosterone, 83 per cent showed subjective, and 24.6 per cent objective, improvement. In another series treated with testosterone, seventy patients received 100 mg. testosterone propionate three times a week. Cases treated for less than one month were not included; 50 per cent failed to return in six months. Doses ranged to a total maximum of 19,000 mg.; improvement was noted with an initial variation of from 500 to 11,400 mg. However, one half the patients who received benefit had achieved it by the time 3000 mg. was given. Palliation extended from two to eleven months but averaged four months. There was objective improvement signified by calcification, reformation of bone, or healing of fractures in 19 per cent of those with osseous metastases. Of those with soft-part lesions, 15 per cent regressed; 26 per cent showed no change; 48 per cent progressed; 11 per cent were inconclusive. Of ten lymph-node metastases, three decreased in size; and of twenty-seven cutaneous metastases, four decreased. New osseous lesions appeared in four of sixteen patients while under treatment, and metastases in soft parts appeared in five of twenty-two who had previously had osseous metastases only. In one patient with multiple skeletal metastases,

one lesion was observed to heal although others progressed. Good results could be reported in 17 per cent of premenopausal patients and in 22 per cent of postmenopausal. Symptomatically, forty-four of fifty-eight patients had relief of pain for variable intervals.

It was reported that seventeen of seventy cases showed an increase of hemoglobin of 26 per cent. In some cases, it increased 16 to 20 gm. There was an increase of bone-marrow activity, and in the red-cell–myeloid ratio. These patients did not have pulmonary metastases. Some cases were carried on large doses for one to two years, and then began to show progression that was not halted by further testosterone treatment. Of a total of six cases, four showed objective and symptomatic improvement on cessation of the testosterone treatment, and it was speculated whether the tumor might not have become adapted to testosterone and then be controlled again by a shift in endocrine balance on withdrawal of it.

In the discussion on estrogens, it was brought out that 20 per cent of patients with untreated carcinoma of the breast are alive after five years and that all figures must be referred to this fact. On dosages of 15 mg. stilbestrol a day, symptomatic relief developed slowly. In untreated primary carcinoma, there were 51 per cent local regressions with stilbestrol; in those with local recurrences following surgery, 46 per cent. Where a combination of surgery and roentgen rays had been employed, the regression rate was 22 per cent. With pulmonary metastases, 37 per cent of the patients felt better and 42 per cent showed roentgen-ray evidence of regression. With bone metastases, there was subjective improvement in 57 per cent and objective improvement in 30 per cent. Calcification of osseous lesions has been observed in some cases only after months of treatment. In general, with estrogens, the older age group gave the better response. Excellent results were observed in a group whose median age was 69 years and good results in one with a median age of 64 years. In the premenopausal group, the development of breast cancer may be accelerated by the use of estrogens, but estrogens gave the best general results in all categories. In a group of ten young women, treated with

100 to 400 mg. daily, one case was accelerated, three showed improvement, and six showed no results.

Another series of estrogen-treated patients, who were two to twenty-nine years postmenopausal, was reported. The case two years postmenopause showed acceleration. Eight of thirty-five patients showed improvement, particularly those with skin metastases. One of twenty-four primary breast cancers showed regression and two of twenty-two node metastases were improved. Two of six patients showed roentgen-ray improvement in bone metastases. Nearly all were improved in general condition. Pain was relieved in eleven of eighteen, dyspnea in eight of sixteen, and the average duration of improvement was seven months. Vaginal bleeding occurred in six, four of these on the withdrawal of estrogen. Nausea occurred in approximately one third of the cases. Twenty of these patients were treated with stilbestrol, 5 mg., three times daily, ten with ethylene estradiol, four with estrone sulfate and one with 3.5 mg. estradiol benzoate intramuscularly three times a week.

In still another series, less than 50 per cent response was noted with either testosterone or stilbestrol. Stilbestrol was considered dangerous in the premenopausal group and it was stated that the patients long past the menopause gave the best results. Some patients responded to both stilbestrol and testosterone. The average duration of response was ten months. Two cases were reported who had progression following a response of estrogen, showed good brief responses on androgen, and then responded to stilbestrol. Two cases with brain metastasis are alive twenty-one and twenty-three months after having had projectile vomiting and papilledema. These are on androgen. Two cases on stilbestrol have developed rectal carcinomas.

Androgens cause water retention, and edema of the operative arm is often seen. Several hypertensive patients were reported to have developed acute cardiac decompensation on androgen therapy. Stilbestrol produces more gynecological complications with bleeding, cervical erosions, and possibly the development of fibroids. Three males were treated on stilbestrol, one with no

results, one, 78 years old, with a good response; and another with pulmonary metastases and pleural effusions that had required weekly taps, who has required no taps since therapy was started and whose roentgenograms show evidence of regression.

It was emphasized that variations in reported results depend upon the selection of cases for therapy; the importance of correct management when reactivation occurs under treatment was stressed.

Excretion Studies

A quantitative chemical analysis of five abnormal ketosteroids—two gonadal and three adrenal—was reported. These studies are designed to evaluate alterations in the steroid metabolism of the body with carcinoma, and to study the effect of biologically active steroids administered. One abnormal steroid was noted for three years in the urine of a patient before the development of carcinoma of the breast, and it persisted after removal of the tumor (now more than five years later). In carcinoma of the prostate, a marked reduction was observed in steroids present in normal individuals. The excretion of one abnormal steroid metabolite related to an adrenal-cortical-hormone precursor was observed in a very large number of patients with carcinoma but was also observed in other metabolic disturbances. With the injection of testosterone, the excretion of androsterone and its isomer etiocholanolone was greatly accelerated, but following the withdrawal of testosterone, both of these substances fell below normal levels. Progesterone does not change the level of steroid excretion. In pregnancy, androsterone and its isomer etiocholanolone are greatly reduced in the eighth and ninth month, 11-hydroxyandrosterone is absent in pregnancy, and large amounts of pregnanolone are excreted. Thus, pregnancy produces a big shift in steroid metabolism. With the administration of adrenal cortical extract in one case, all steroids were greatly reduced in the urine. A tool is now available to estimate steroid metabolism quantitatively and study its alterations in cancer and after the administration of biologically active steroids.

Experiments were reported in the study of the excretion of

17-ketosteroids by mice. It was observed that the stool of cattle and of mice contains 17-ketosteroids. In two strains of mice reported, ketosteroid excretion was higher when the milk factor was not present and lower when present or induced by injection. This drop was largely in the dehydroisoandrosterone and androsterone fraction. No biological difference is noted between the two groups in time of onset of estrus, but it was suggested that the period of cornification of vaginal smears might be of longer duration in mice in which the milk factor was present.

It was brought out that there is a mechanism for preventing renal excretion of testosterone, perhaps due to a linkage with plasma albumin, as the substance is not filtered in high levels by the glomeruli. It was shown that testosterone has a solubility of 800 γ per cc. in plasma, whereas, in plasma filtrate, its solubility is 1/12th as great. It was observed that there are reducing enzymes in many tissues that act on ketosteroids, and the point was raised that other end products, as yet unidentified, may result when certain enzyme systems are present.

PANEL ON THE LYMPHOBLASTOMAS

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FUNDAMENTAL NATURE OF THE LYMPHOBLASTOMAS

Tissue Culture

The following characteristics have been observed in tissue cultures made from lymph nodes taken from patients with Hodgkin's disease: large multinucleated giant cells, liquefaction, inclusion bodies, and rapid disintegration. According to Rottino, however,

such are not characteristics specific for Hodgkin's disease nor do these necessarily indicate the presence of a virus.

The results of tissue culture differ somewhat between laboratories, but it may be pointed out that different tissue-culture studies are not strictly comparable, since many different factors are involved. The question was even raised whether different types of cells may not emerge from the same mother cell according to the medium on which the material is grown. Hydrogen ion concentration is an important factor. It was suggested that synthetic media of known constitution should be used under standard conditions in so far as possible. Even with these limitations, however, culture studies have been shown to be of value: (1) as a diagnostic adjunct, (2) for histochemical studies, (3) in maintaining cell strains, (4) in studying in vitro carcinogenesis, (5) in studying responses to chemical substances and irradiation, and (6) for single-cell isolation.

Hoster tentatively proposed three types of Hodgkin's-disease tissue on the basis of culture: Type I is the most common and is characterized by free cell growth. It consists principally of macrophage-type cells and reticulum cells containing a neutral-red rosette in the Hof of the nucleus, and refractile granules that stain with Sudan IV. Type II, also a free cell type, consists mainly of abortive, rounded, fibroblast forms. Type III, a syncytial form of growth, is made up mainly of fibroblasts. The question of whether such growths were the result of pure chance or not was tentatively answered by the observation that in three cases of Hodgkin's disease, cultures of repeated biopsies resulted in the same type of growth as that from the initial biopsy. To date, however, no correlation has been made between these types and the clinical types of Hodgkin's disease. Certain very preliminary observations have suggested that Type III may be associated with the development of edema resulting from lymphatic obstruction. It is suggested that the available evidence indicates the presence of a substance or factor in Hodgkin's-disease serum that is not present in normal serum. Reference was made to the changes in the morphology and the growth pattern presumably due to the presence of this abnormal factor in the serum.

'Gey stressed the value of motion-picture observations of living cells from tissue cultures as a means of identifying blood-cell types. The characteristic activity of monocytes and macrophages is well recognized. Certain criteria have been established for the recognition of lymphocytic and myelocytic cells. It is admitted that the final proof of their identification is still lacking. The question is still unsettled whether or not one cell type may change in tissue culture to another, such as the transformation of lymphocytes into monocytes or macrophages. Also, it has not been proved that macrophages can change to fibroblasts and back again as claimed by some. Until cultures of single blood cells can be successfully produced, the question will probably remain unsettled.

'Appropriate culture procedures permit the growth of certain blood-cell tumors for a number of years. This has been accomplished with a spontaneous lymphoblastic and a spontaneous myeloblastic sarcoma of mice and with a dibenzanthracene-induced lymphosarcoma of the mouse. Continuous cultures of a monocytic sarcoma of the mouse have also been maintained. Several observers have maintained that mesenchyme may supply a factor or factors necessary for the growth of lymphocytic and of myeloblastic sarcomas of the mouse. However, W. W. de Bruyn has developed a strain of altered lymphoblasts from a lymphoblastic sarcoma of the mouse that will now grow independently of normal mesenchyme. So far, all preliminary attempts to demonstrate the transplantability of these cells have failed, yet the original lymphoblastic sarcoma could kill a mouse within ten days.

'Continuous cultures of normal blood cells have been uniformly unsuccessful with the exception of chick macrophages. These have been maintained in continuous cultures by several investigators. Gey et al., in addition to successful results with chick macrophages, have observed survival of normal human lymphocytes in four-month-old cultures of human fibroblasts derived from lymph nodes. Inability to grow the normal prototype of the blood-cell tumors that can be maintained in cultures has not been explained. This points directly to the need for further experiments aimed at establishing the differential requirements of normal and malignant cells of the same type maintained in vitro. Microbiological

assays and other biological studies of the rate of disappearance or utilization of various essential constituents of the culture media should be attempted. At the present, it is known that most of these cells can be maintained in plasma or serum.'

Osgood's technique of marrow culture permits one to obtain quantitative data on cells in suspension—such information as the number of cells in mitotic division, the influence of colchicine, and so on. This method, however, has the limitation that growth can be obtained only over a very short period of time, approximately forty-eight hours. In a few instances, mitosis has been observed at eighteen to twenty days, and motile cells have been present for as long as fifty-two days. This technique has revealed that cells from patients with acute leukemia differentiate no further in culture than in the human subject, an observation that implies that the factor governing the abnormal growth of the cell is intracellular rather than environmental.

Chick-Embryo Studies

Chick-embryo studies have demonstrated that heterologous tissues can be grown on the chorioallantoic membrane. However, explanation of human leukemias, mouse leukemias, mouse lymphosarcomas and tissue from Hodgkin's disease, has been consistently unsuccessful. Hoster and Karnofsky found that the mortality rate of the chicken embryo is increased by the implantation of such tissues, particularly that of Hodgkin's disease. Karnofsky has noted edema of the embryo to occur in 30 to 40 per cent within the first five days of implantation, but he does not regard this as a reaction specific to Hodgkin's disease. The question was raised whether this reaction might represent the effect of a heterophile reaction induced by the Hodgkin's disease tissue and often present in these patients.

Chick-embryo studies have revealed no evidence indicating the presence of an infectious agent in Hodgkin's-disease tissue. Failure to receive an adequate blood supply was suggested as a reason for the failure of leukemic cells to grow in embryo cultures. Certain reticulum-cell sarcomas do, however, grow in chick embryos. The question was raised whether or not antibodies present in the

embryo might not affect the susceptibility of the embryo as a growth medium. It was pointed out that the chick embryo is not a standard animal of consistent nutritional background.

Thiersch described failure to secure growth in the chorioallantoic membrane after the injection of leukemic cells but indicated that in those embryos that hatched, osteopetrosis, a form of fowl leukosis, appeared in a high percentage within six to eight months. The offspring of these were free from petrosis. Repetition of the experiments in 1-day-old hatched chicks yielded a low percentage of osteopetrosis. Subsequent passages with heparinized blood were easily established in 1-day-old chicks. The time of development of the osteopetrosis was shortened to three weeks by three passages. Lymphoid leukemia appeared with the osteopetrosis in the fourth passage. Control inoculations from various bone tumors were negative. Thirteen passages and thirteen bioassays were made of cells from two strains of AK mouse leukemia, which had been injected intravenously in 9-day-old chick embryos. These all proved negative, even though the leukemic material from the original AK donor mice could be detected on the fourth day after inoculation. There was general agreement that the negative results could not likely be attributed to the toxicity of the heparin used in these experiments.

Microscopy

Electron microscopy, which permits resolution down to 10 to 20 Angstroms has revealed the presence of agents within the cells. The technique, however, is extremely difficult to use in the study of cells, for the preparations must be thin and dry, and the stains must be adequate. The nucleus of cells is too thick to be studied. Several viruses have been visualized in cells by this means, but it cannot be said that a virus will necessarily be identified by this method.

According to Zanes, phase microscopy has simplified the demonstration, in malignant cells, of an increase in the number of nuclei and of nucleoli, of variation in the size and shape of the nucleoli, and of refractile bodies with varying sizes and shapes and frequently grouped in a rosette in the Hof of the nucleus within the

cytoplasm. Differentiation between chromatin clumping and nucleoli is made easier by this technique, and fine points in nuclear detail are demonstrable. In general, better resolution of all formed elements is possible than can be obtained with the ordinary microscope. Further improvements are needed, however, and the suggestion was made that phase plates with less contrast than those generally used might be better. Rottino feels that phase microscopy of tissue cultures is feasible. With it one observes fine cytoplasmic detail in reticulum cells and fibroblasts as well as good nuclear detail in fibroblasts and lymphocytes.

Ultracentrifugation

Ultracentrifugation is being used for the study and comparison of macromolecular particles obtained from neoplastic and normal tissues. The type of centrifuge employed for this purpose depends to a great extent upon the initial quantity of material and the desired purity of the product. Among the common types of centrifuges in general use are the spinning-top, the hatch-bowl, and the angle-head types. In the first of these, effective gravities as high as 100,000 are obtained. This centrifuge has the advantage that very small quantities of material can be processed effectively. The Sharples ultracentrifuge offers an effective gravity of 62,000 and the advantage that relatively large amounts of material may be centrifuged at once. The high-speed attachment of the International type I SB centrifuge has an effective gravity of about 25,000.

The factors involved in the segregation and visual study of macromolecular particles are: (1) the ionic strength of the dilutant and the hydrogen ion concentration; (2) the extraction temperature and time of extraction; and (3) the method of centrifugation. Technical problems encountered are: (1) maintenance of particle dispersion; (2) fractional separation of particles; (3) removal of fat; (4) prevention of gel formation; and (5) staining of particles. Certain techniques for the segregation and visual study of tumor viruses reported in the literature have been found to be inadequate in the study of human lymph-node tissue. Schoenbach stated that, in 1940, during studies with the ultracentrifuge for analysis of Brown-Pearce carcinoma, it was found that disruption of cells

with high pressure (nitrogen) followed by sudden release of pressure would result in a minimum of denaturation and artifact. This technique may be used for preparation of cytoplasmic components for the ultracentrifuge or electron microscopic studies.

Transplantation Studies

Furth stated that the best evidence on the fundamental nature of the lymphoblastomas has been obtained from transplantation studies in mice. These indicate that the lymphoblastomas are truly neoplastic. All of the tumors that occur in mice spontaneously have been transplanted successfully, and the laws of transplantation seem to be the same as those that govern other neoplasms. These are in part genetic and in part environmental. Most of the available evidence indicates that the conclusions derived from these studies of transplanted tumors in mice are applicable to human lymphoblastomas. Nevertheless, unsuccessful attempts at transplantation must be explained. Small lymphocytes are more predominant in animals than in man and yet the small-cell–type leukemias have not been successfully transplanted in animals. It was pointed out that transplantation studies can be of use in furnishing a uniform cell population over extended periods of time for precise biological, chemical, and physical studies designed to determine the characteristics of the leukemic cell.

Law stated that in animals in which a high degree of homogeneity is obtained by continued brother-sister matings (mice, rats, guinea pigs), leukemias are transplantable and follow the laws that govern the transplantation of tumor types. The small-lymphocyte types that have been observed have occurred in heterogeneous animals and one would not expect transplantation to be accomplished (as in human cases).

Transplantation of many strains of leukemia has been useful in studying chemotherapeutic agents, at least twelve of which have been found to be effective against mouse leukemia. The results vary with the strain of leukemia used. This is probably a reflection of fundamental biochemical differences in the strains of leukemia. In one strain of lymphoid leukemia AK4, 4-amino-N^{10}-methylpteroylglutamic acid has been found to increase the survival time

from fourteen to about thirty days and the leukocyte count could be kept below 32,000 per cu. mm. even though the mice eventually died. 2,6-Diaminopurine has also been found to increase the survival time. However, the in vitro incubation of tumor cells with very high levels of the folic acid antagonist for one hour did not destroy the neoplastic character of the cells as measured by in vivo assay. Of 690 chemical substances assayed for antileukemic activity in mice, only twenty-one have been found to be active. The question was raised whether spontaneous leukemia rather than transplanted leukemia might be the better test type, since transplanted leukemia is, in reality, an in vivo culture.

Law pointed out that arsenic was effective in transplanted chronic myeloid leukemia only when given in the preproliferative phase. When given thereafter, it was relatively inactive. It follows, therefore, that this type of assay is not strictly comparable to the assay of chemotherapeutic agents in human leukemia. In spite of this limitation, however, the method has some value since agents that have been found to be effective by mouse assay have been the ones that prove to influence human leukemia.

Spontaneous Lymphoblastomas in Various Species

Spontaneous lymphoblastomas have been observed in many species other than mice: cattle, horses, sheep, goats, swine, dogs, rats, guinea pigs, pacas, and opposum, as well as canaries, parakeets, cockatoos, pheasants, turkeys, and chickens. The incidence among chickens is higher than in any other species and may be as high as one out of three in a completely studied population.

In chickens, various forms of lymphoblastomas may be observed, including the neural, visceral, ocular, and osteopetrotic lymphomatoses, and the more rare types, erythroblastic and granuloblastic leukoses. It has long been known that erythroblastic and granuloblastic leukoses can be transmitted with filtrates. Recently, it has been established that visceral and osteopetrotic lymphomatoses may also be induced by a filterable agent or agents that have some of the properties of the viruses. Filtrates are capable of producing tumors in chicks in about ninety to 150 days. There is evidence that visceral lymphomatosis may be transmitted through

the egg from apparently normal dams to their offspring. Extracts prepared from the tissues of apparently normal chick embryos, when injected into susceptible chicks, will produce lymphomatosis in ninety to 150 days. The disease also appears to be transmitted from infected to noninfected chicks during the hatching and brooding period.

Birds appear to be delicate histological indicators of the precancerous stage of lymphomatosis. Lymphoid foci in duck pancreas show invasion and destruction of acinar tissue, plugging of blood vessels, destruction of the islets of Langerhans and some destruction of the intrinsic nerve ganglia of these organs. These histological changes may represent early lesions of lymphoblastoma.

Forbus discussed a disease observed to occur spontaneously in swine that more closely resembles Hodgkin's disease than any animal disorder hitherto reported. Granulomatous lesions have been found in the spleen, lymph nodes, and bone marrow. In the early stage, there is proliferation of the reticulo-endothelial cells, marked variation in the size and form of the cells, and giant cells similar to the Reed-Sternberg type. The lymph nodes show areas of necrosis, and in the late stage, there is marked scarring and extensive collagen deposition. In the final stage, the lesions are composed almost entirely of scar tissue with scarcely any cells. Cirrhosis of the liver is often found at the same time. Thus, the histological picture is similar to that of Hodgkin's disease in man. However, attempts to transmit human Hodgkin's disease to hogs have not, so far, been successful.

The studies of Vallee have revealed that leukocytes, when compared with erythrocytes, contain twenty-five times as much zinc per million cells. About 75 per cent of the whole-blood zinc content is found in erythrocytes, about 3 per cent in leukocytes, and 22 per cent in the plasma. Erythrocyte zinc appears to be bound to carbonic anhydrase. The function of the zinc in the leukocytes is not understood. In the leukocytes of leukemia, the zinc is decreased to 10 per cent of the normal and increases following effective therapy of the leukemia.

The administration of radioactive zinc to a patient with chronic

myelogenous leukemia revealed that only about one tenth of the radioactive zinc was taken up in the whole body as compared with normals. The administration of stable zinc to a patient with chronic myelogenous leukemia caused a drop in the leukocyte count, but the further administration did not continue this effect; nor did the administration result in an increase in the zinc content of the leukocytes. Ross suggested that the low zinc content might be due to the immaturity of the cells, since mature lymphocytes have been found to have a higher zinc content than immature ones.

The observation that in a tumor produced in mice by painting the epidermis with methylchloranthrene, there was a decreased content of zinc, iron, calcium, and copper suggests that there may be a change in the metal topography of neoplastic cells, as compared with the normal.

Bierman proposed a formula relating the leukocyte level of the blood to a balance between proliferation of leukocytes and their rate of removal. The formula is:

$$L = P + \frac{\text{Duration of life}}{2} - (S + B)^x$$

where L is the level of circulating leukocytes, P the rate of proliferation, S the storage of leukocytes, and B the breakdown of leukocytes. By subjecting heparinized blood to ultrasonic vibration at 635 kilocycles per second, a logarithmic destruction curve was obtained, and the average half-life of normal leukocytes found to be about one to six minutes. The leukocytes of young and elderly individuals were found to possess shorter half-life periods than those of individuals in the middle-age group. Lymphocytes and neutrophils were found to have about the same half-life. The half-life of cells from patients with chronic lymphatic leukemia was, surprisingly, found to be increased to as much as eighteen to twenty-two minutes. Thus, the inference may be made that leukemic cells may have a longer circulating life owing to their increased resistance to destruction. After treatment the life span of leukemic cells was found to have decreased but never to the level found with normal white cells. The transfusion into normal

volunteers of up to sixty billion leukocytes from patients with chronic leukemia resulted in an increase in the circulating leukocyte count of only about 2000 to 3000, and even this small increase was not sustained. This raises the question of whether or not, in leukemia, the tissues had lost their ability to take up and destroy leukocytes. It is therefore suggested that the ability to remove cells from the circulating blood may be at least as important as to proliferate them. If this is substantiated, it may shift the emphasis of chemotherapy away from purely destructive agents to those that may enhance the controlling mechanism.

Heinle described similar types of experiments conducted in rabbits. When leukocytes obtained from the rabbit peritoneal cavity were transfused back into the blood stream of the rabbit, leukopenia, rather than leukocytosis followed. This leukopenia persisted for several hours and was followed by leukocytosis. A saline extract of the leukocytes was found to have a similar action. When leukocytes labelled with radioactive phosphorus were injected, it was found that the radioactivity (and the leukocytes) was concentrated in the lungs. Later, the radioactivity shifted from the lungs to the liver and the spleen. However, no radioactivity was found in the blood during the period of leukocytosis, thus suggesting that the leukocytosis that follows the initial leukopenia was due to a release of new leukocytes from the bone marrow rather than release of the transfused leukocytes from the lungs. It was further observed that the injection of large quantities of leukocytes by this means resulted in death. Part of this toxicity may be due to the thromboplastic content of the leukocytes, but not all could be explained by this means. The leukopenia could not be prevented by antihistaminic compounds.

Miller reported that keto acids obtained from the urine of patients with leukemia, when injected into guinea pigs, produces a tissued myeloid response. Hydroxy acids when so injected caused a lymphoid response. When the two types of acids were given together, the tissue response resembled that seen in Hodgkin's disease. Likewise, the injection of urine from patients with Hodgkin's disease into guinea pigs resulted in a Hodgkin's-type reaction.

Meyer stated that he has been able to confirm Miller's work and

that a lymph-node culture is now being used for a simplified assay.

Custer, from the histological examination of the tissues of 1300 patients with lymphoma, including 700 cases of Hodgkin's lymphoma (many of which had multiple biopsies), has concluded that there is a remarkable fluidity in the histological pattern of the lymphomas, and that there is a very close relationship between them. In the Hodgkin's group, the transformation of one type to another was observed in 40 per cent of the cases. A pure histological form was seen in only 20 per cent. Hence, the ostensibly separate lymphatic tumors were regarded as variants of a single neoplastic entity. Clinical behavior could be correlated with the predominant histological pattern in only a very general way, and rules regarding a given type of tumor could not be applied to an individual patient with any degree of certainty. It was suggested that the tumor be classified as a malignant lymphoma, and the subgroup indicated as: follicular type, reticulum-cell type, and so on.

Forbus called attention to the limited capacity of the reticuloendothelial system to react to various agents. Since it is unlikely that all these reactions represent the manifestations of but a single disease, even though they may be morphologically indistinguishable, the limitations of the purely morphological approach become evident.

Osgood, in defense of the morphological approach, pointed out that malignant cells show considerable variation in size, in large nucleoli, and in the depth of staining of the nuclear chromatin. Morphology is a necessary prerequisite to the chemical approach, since it is necessary to know what cell type is being worked with chemically.

Polycythemia Vera and Leukemia

Of 125 patients with polycythemia vera studied by Dobson, a majority were found to have leukocytosis. In 40 per cent, there was a leukemoid blood picture; 70 per cent of the leukemoid reactions disappeared after therapy. The disease was indistinguishable from leukemia in about 5 per cent of the patients. This leukemia was stated to be either acute or subacute, and of the granulocytic type. Several peculiarities in the type of leukemia were noted. The ques-

tion was raised whether the development of leukemia was related to therapy and whether the incidence of leukemia would vary with the type of therapy used.

Hall noted that leukocytosis, splenomegaly, thrombocytosis, and leukemoid reactions are characteristic of polycythemia vera, but that they are not seen in secondary polycythemia. Hyperplasia of all elements in the marrow is noted in the former but not in the latter. Of 163 patients with polycythemia vera, splenomegaly was noted in two thirds, leukocytosis in 80 per cent, and a leukemoid reaction in approximately one third. These patients had received neither P^{32} nor roentgen-ray therapy. In 12 per cent of thirty-five cases of polycythemia vera dying from causes that could be determined, leukemic blood pictures were found that were indistinguishable from those of chronic myelocytic leukemia. The necropsy changes were also compatible with those of leukemia. The question was raised as to whether polycythemia vera represents a mild form of leukemia or whether leukemia is simply a complication. A few cases have been reported in the literature in which acute myeloblastic leukemia developed in patients with polycythemia vera, especially after roentgen therapy. However, the development of polycythemia vera in patients with leukemia is of rare occurrence. The survival time of 101 patients with polycythemia vera treated principally with phenylhydrazine and/or venesection was as follows: Three patients, twenty years or more; ten patients, fifteen to nineteen years; fourteen patients, ten to fourteen years; forty-two patients, five to nine years; and thirty-two patients less than five years; forty-five of the 101 patients still were living at the time these data were compiled. It was pointed out by others that polycythemia vera is a disease comparable to a form of fowl leukosis (erythroleukosis). There is no adequate evidence to believe that there is a separate agent causing erythroblastosis and granuloblastosis in chickens.

White commented that adrenocortical extract, pituitary adrenocorticotropic hormone, and adrenocortical steroids, especially the 11-oxy compounds, decrease lymphoid-tissue mass. Epinepherine is capable of producing a similar change via nonspecific stimulation of adrenocorticotropic-hormone release. Histological examination of the nodes reveals a washing out, degeneration, pyknosis, bud-

ding, and shedding of the cytoplasm of the lymphocytes. It has been both claimed and denied that these changes can be produced in vitro. These just-mentioned substances also produce an acute, absolute, profound lymphopenia. With the lymphopenia, there is an increase in some of the globulin fractions in the serum of mice and rabbits, but this has not been found to occur in rats and human subjects. The demonstration of a rise in antibody globulin depends upon the time interval between the administration of antigen and hormones. It has been demonstrated that lymphocytes contain γ globulin after immunization, but this does not mean that the lymphocyte is the site of the synthesis of antibodies. It may indicate that antibodies are formed in the parent cells of lymphocytes and merely passed on to the lymphocytes. Adrenalectomized animals are capable of synthesizing antibodies, although at a reduced rate. In the adrenalectomized animal, both the rate of synthesis of antibodies and their destruction decrease, with the result that the circulating antibody content may not be substantially altered from the normal. Evidence is available to indicate that all lymphoid structures may not synthesize antibodies. Adrenal cortical steroids may stimulate their dissolution. The natural resistance of the host may be an important factor in the response of neoplastic lymphoid tissue to therapy.

Dobriner raised the question whether steroid metabolism is altered in diseases of the lymphatic system, and whether or not such diseases can be influenced by steroid hormones. In this regard, compound E should be tried and further studies with adrenal cortical extract are indicated. It remains to be demonstrated clearly whether or not steroid hormones are involved in the production of leukemia. It has been observed that 11-hydroxy-etiocholanolone is excreted in the urine of patients with chronic lymphocytic leukemia but not in the urine of patients with myelocytic leukemia. These observations suggest that there is a disturbance in the production of adrenal cortical steroids in lymphocytic leukemia indicating an adrenal dysfunction. The endocrine balance should certainly be studied in more detail.

Schoenbach commented that there is a difference in the toxicity of 4-amino-pteroylglutamic acid in male and female mice and rats.

The administration of estrogenic hormones did not abolish this differential toxicity. The action of the estrogens both proliferative and atrophic would appear to be blocked by 4-amino-pteroylglutamic acid. This blocking appears to be on the end organ. The action of androgen was not affected by 4-amino-pteroylglutamic acid. Follicle stimulating hormone, 17-ketosteroid, 11-oxysteroid, and estrogen excretion is not affected by this folic acid antagonist.

The question was raised as to whether there was any fundamental difference between the nature of lymphomas and other types of tumors such as carcinoma. Whether Hodgkin's disease represents a unique disease separate from the other lymphomas was also asked. Conclusive answers are not available. It was suggested that the more divergent the approaches and the more varied the concepts, the more likely is there to be progress.

Various possible causative agents or factors in the development of lymphomas were listed by Craver as (1) exposure to benzol and various other solvents, (2) the production of endogenous carcinogenic substances, (3) sulfonamides, (4) possibly myelokentric and lymphokentric acids, (5) radiation, (6) viruses, (7) chronic infections, (8) heredity, (9) allergy, (10) lack of immunological response, and (11) hormonal factors.

Shear divided chemotherapeutic agents into three groups: (1) those that have given negative results in spite of various claims—under this grouping were included H 11, K-R, ensol, chymotrypsin, and biotin; (2) those agents that have not yet been established as being of value or about which there are conflicting reports —this group includes crude penicillin and related antibiotics, dyes, and desoxypyridoxine; (3) those agents that have been demonstrated to produce a partial change in at least one type of temporary malignant growth. In this group were included benzol, Fowler's solution, nitrogen mustards, urethane, bacterial polysaccharides, and the pteroylglutamic acid antagonists. A relatively large number of classes of compounds, some members of which are capable of damaging transplanted tumors in mice, have been found. The outlook seems promising because there is an increasing number of compounds worthy of the attention of investigators.

Clinical Aspects of the Lymphomas

The case history of a patient exposed to *benzol poisoning* was reported by Fienberg. The histological picture revealed multinucleated giant cells, not unlike those of the Reed-Sternberg type of Hodgkin's disease. A theory explaining the various types of leukemia arising in benzol poisoning on the basis of a release mechanism acting in localized areas on preneoplastic cells was presented.

The cause of *fever* in Hodgkin's disease is unknown. Antibiotic therapy is successful in a few patients with obvious infections. The fever responds rapidly to the administration of nitrogen mustard. No correlation has been observed between the histological pattern and the occurrence of fever.

The cause of the *anemia* in the lymphomas is unknown. In a few patients the anemia is obviously hemolytic, but in the majority, no adequate explanation is available. The possibility that the anemia is due to crowding out of functional marrow tissue by the invading cells was discussed. The majority were of the opinion that this explanation can be true in only an occasional patient. There is little correlation in most patients between the degree of infiltration and the degree of anemia. Hypersplenism, the sludge phenomenon, division of metabolites to rapidly growing neoplastic cells, and protein deficiencies, are among the explanations which were offered. It was noted that the anemia is chemically distinguishable from the anemia of infection in some but not all instances.

Only a few *biological and chemical differences* have been observed between normal and neoplastic leukocytes. White commented that leukemic cells have a higher rate of anaerobic glycolysis than do normal cells. Vallee has observed that the zinc content of leukemic cells is lower than in normal leukocytes. Hoster has observed that when neonatal lymph nodes are cultured with serum from patients with Hodgkin's disease, an abnormal type of growth results. When tissue from Hodgkin's disease is incubated with serum from the same disease, there is some degree of change in the type of growth, but not so much as when normal tissues are cultured with Hodgkin's-disease serum. Thus, normal cells appear to be more responsive to abnormal serum than are abnormal cells in this case.

Furth pointed out that leukemic lymphocytes grow in tissue cultures, whereas normal lymphocytes do not. He suggested that comparative studies be made on normal and leukemic leukocytes under comparable conditions measuring viscosity, motility differences, phagocytic ability, chemotaxis, bactericidal activity, bacteriostatic activity, formation of antibodies, antigenicity (by modern immunological studies), genetic and antigenetic properties, ribonucleic acid and desoxyribonucleic acid content, histochemical and chemical (enzymatic and other) assays, and response to various chemical agents, etc.

Variations in Course of Lymphoblastomas

That the lymphomas present insensible gradations between benign infectious and reactive processes on the one hand and frankly malignant processes on the other was proposed by Craver. Identical histological pictures may vary in their response to specific therapy. Patients with the supposedly very malignant sarcomatous type of Hodgkin's disease may live much longer than three years, a time limit set by Jackson and Parker. Thus, there appears to be little correlation between the histological pattern and the course of the disease. Follicular lymphoma may blend into diffuse follicular nests and then into reticulum-cell sarcoma. Lymphosarcoma frequently begins or seems to begin in a local focus and then becomes generalized. The differentiation between a benign and a malignant orbital lesion may be quite impossible. Reticulum-cell sarcoma of bone should be regarded as simply a type of local onset of essentially the same disease as reticulum-cell sarcoma of other sites of initial appearance. Mycosis fungoides, with its peculiar clinical behavior and rather long survival time, may, in the later stages, be histologically indistinguishable from reticulum-cell sarcoma. True mycosis fungoides has not been observed to change into leukemia or Hodgkin's disease. Rarely, patients with Hodgkin's disease give a history of nodal enlargement or other manifestations for a period as long as thirty-five years. A slow transition from a precancerous to a cancerous state is suggested. Boeck's sarcoid has been observed in a few cases to show transitions into reticulum-cell sarcoma, or Hodgkin's disease.

Treatment

Craver distinguished three classes of disease: (1) disease that seems to be localized and in which aggressive therapy is extremely worth while, (2) disease that has begun to spread, and (3) generalized disease. In the last which includes all leukemia, therapy is only palliative.

Radioactive Agents

More attention should be devoted to the localization of roentgen-ray therapy and in this respect round or oval ports might be used. This may be very important when patients are treated for long periods of time in more than a few fields. For deep lesions, supervoltage is indicated, since there is less effect on the skin and less radiation sickness. The problems of acquired roentgen-ray resistance were discussed and the value of surgical treatment was stressed. The pros and cons of the diagnostic use of roentgen rays for the differential diagnosis of mediastinal tumors was also considered. There was no general agreement on this subject.

Of 242 patients with lymphosarcoma, treated by Lenz, 169 received roentgen-ray therapy; thirty of these were well at five years. Of fifty-five that received surgical as well as roentgen-ray therapy, twenty were well after five years. However, other lesions appeared in these twenty patients. In his opinion, surgery would seem to have little place in the therapy of lymphosarcoma, other than lymphosarcoma of the bowel. Results of roentgen-ray therapy alone are as good as those of roentgen-ray therapy plus surgery. The value of roentgen-ray therapy varies with the histological diagnosis, as evidenced by the following figures:

	SYMPTOM-FREE	
	5 years	10 years
Reticulum-cell	12.3%	14.5%
Lymphocytic	23.1%	19.6%
Giant-follicular	42.0%	33.3%

Local Persistence or Recurrence

Reticulum-cell	64%	Tumor dose less than 500 r.	92%
Lymphocytic	51%	500 r = 1999 r	60%
Giant-follicular	31%	3000 r or more	20%

For aggressive therapy as much as 5000 r may be given. Smaller masses have been found to recur less frequently than larger masses. Survival depends upon the primary site and the degree of dissemination. Women have been observed to do better than men, whereas children do poorly. The results of this type of therapy in Hodgkin's disease have not been so good as in the lymphosarcoma group, the five-year-survival rate being 12 per cent. The prophylactic irradiation of mediastinal and retroperitoneal areas may be of some value.

Therapy with P^{32} has its limitations since the irradiation of the bone marrow is rather severe. Treatment with this agent has been discontinued in Hodgkin's disease and in acute leukemia, since the results are not encouraging. The results in chronic granulocytic leukemia would appear to be about equal to those obtained with roentgen-ray therapy. Occasional favorable results may be seen in patients with multiple myeloma. P^{32} is a valuable agent for the therapy of polycythemia vera.

Hall has observed the appearance of chronic leukemia in one patient of 172 treated with P^{32}, whereas the incidence of chronic leukemia in patients with polycythemia treated with venesections and/or phenylhydrazine is about 12 per cent. Acute leukemia has been observed to follow P^{32} therapy in four patients of 172 with polycythemia vera, whereas the incidence of acute leukemia following venesections and/or phenylhydrazine is less than 1 per cent. Acute leukemia has been observed as a terminal phenomenon in 78 per cent of twenty-two patients with chronic myelocytic leukemia treated with this agent. The incidence of acute leukemia in the terminal stage of chronic myelocytic leukemia treated with roentgen-ray therapy has been observed to be about 30 per cent. This raises the question of whether radioactive-phosphorus therapy precipitates the acute phase of leukemia. However, since the complications of polycythemia vera, such as thrombosis and hemorrhage, are greatly reduced by this form of treatment, its use would seem to be indicated. Ross has observed the development of massive splenomegaly in patients with chronic leukemia treated with P^{32} for long periods of time, but Hall and Craver commented that this feature is not infrequently seen in cases treated with roentgen

rays. The incidence of acute leukemia developing in Ross's series has been only 15 per cent.

Nitrogen Mustards, Urethane, and Other Agents

Stock discussed screening procedures using mouse leukemia, solid tumors, tissue cultures, and egg cultures. Inhibition of the growth of sarcoma 180 by the injection of various agents one day after transplantation of the tumor in mice has been a useful screening procedure. Multiple approaches using different tumors under different conditions would seem to be advantageous. Investigations should follow along lines indicated by the clinical activity of such compounds as the nitrogen mustards, urethane, and vitamin antagonists. Results so far have indicated that derivatives of urethane in general are less active than urethane itself. Studies have been conducted using 4-amino-pteroylglutamic acid, 4-amino-N^{10}-glutamic acid, 4-amino-pteroylaspartic acid, and 2,6-diaminopurine. The results would seem to indicate that these agents are more effective against tumors of lymphoid origin. Burchenal stated that his group has tested the following groups of compounds: seventy different pyrimidine compounds, twenty-five pteridine compounds, eleven purine derivatives, and twenty-eight different pteridine–para-aminobenzoic acid–amino acid residue compounds. Only the last group of compounds were found to be active. Karnofsky pointed out that urethane and the folic acid antagonists are toxins to both normal and neoplastic bone marrow. There is a spectrum formed by various tissues in regard to their sensitivities to these compounds. The tumor cells of Hodgkin's disease would appear to be more sensitive than normal cells. It was pointed out that roentgen rays produce a slow and prolonged type of damage, whereas nitrogen mustards produce rapid damage with rather rapid recovery. Some tumors are more sensitive than are normal marrow cells. There appears to be a direct relationship, which is quantitive in nature, between roentgent-ray dosage and nitrogen-mustard dosage.

Gelhorn compared the results in a group of patients with visceral Hodgkin's disease treated with roentgen rays alone and with alternate courses of nitrogen-mustard and roentgen-ray therapy. The tentative conclusion has been reached that nitrogen mustard

has a significant effect on mortality during the first year after the onset of visceral Hodgkin's disease. Of those patients treated with roentgen rays alone, 52 per cent were dead at one year. Only 29.5 per cent of those treated with alternate courses of nitrogen mustard and roentgen rays were dead at one year. Patients with reticulum-cell sarcoma do not appear to respond favorably to nitrogen-mustard therapy, nor do patients with lymphocytic lymphosarcoma. Favorable results have been observed in 32 per cent of the patients with chronic myelogenous leukemia treated with nitrogen mustard. Results have been less favorable in patients with chronic lymphocytic leukemia and uniformly poor in patients with acute leukemia. Using a derivative of nitrogen mustard, SK 136, Burchenal has noted good results in chronic myelogenous leukemia but universally poor results in acute leukemia. The paradoxical situation that urethane is more effective against normal lymphoid tissue than against normal myeloid tissue—yet more effective in chronic myeloid leukemia than in chronic lymphoid leukemia—was brought to the attention of the group by Kirschbaum. Osgood has noted a good correlation between the effects of nitrogen mustard in bone-marrow cultures and its clinical activity. Cells capable of mitosis are inhibited by nitrogen mustard as well as are those not capable of mitosis. Ionizing radiation appears to act by preventing cell division, whereas nitrogen mustard appears to be an all or none affair, and the effect appears to take place during the first circulation time since little effect has been observed in marrow cultures with the concentration of 1:2 million.

It was agreed by all that nitrogen mustard has an established place in the therapy of Hodgkin's disease. The rate of recovery from the effects of nitrogen mustard are much more rapid than from roentgen rays.

Rundles has observed beneficial effects from nitrogen-mustard therapy in seven patients with multiple myeloma. These beneficial effects include an increase in skeletal calcium, a decrease in serum gamma globulin to nearly normal, an increase in serum albumin, an increase in hemoglobin in blood, and a decrease in the proteinuria.

Many variations in the urethane molecule have been made by

Skipper and his group. Most of these derivatives have been found to be less active than urethane or even totally inactive. There may be a synergistic action between urethane and nitrogen mustard in leukemia in mice, since the life span of mice treated with both agents is considerably increased beyond the life span of mice treated with either agent alone. Using urethane labelled with C^{14} in the carbonyl position, it has been found that 90 per cent is expired through the lungs in twenty-fours, 2 to 3 per cent appears in the urine, and the remainder stays in the body. Biochemical studies are now under way to determine if alterations in chromosomal elements take place.

Heinle has observed that folic acid antagonists have limited value, and that the most remarkable remissions have been seen in acute leukemia in children. Continuous therapy in doses of 1 mg. per day per patient, for periods of ten to fourteen days is desirable. Derivatives of 4-amino-pteroylglutamic acid, which are less toxic, have also been found to be less effective. These antagonists have been observed to cause only temporary remissions, and since an alteration in pteroylglutamic acid metabolism is probably not the fundamental defect in leukemia, these drugs will probably have limited value. Thiersch is of the opinion that the action of the antagonists is essentially that of producing a deficiency of pteroylglutamic acid. He has noted some correlation between the duration of therapy and the length of remission. Hall confirmed Heinle's statement that remissions are more common in children than in adults. Forty per cent of children treated with 4-amino-pteroylglutamic acid have shown remissions, whereas remissions have been observed in only 5 per cent of the adults treated. Schoenbach stated that these antagonists may block compounds other than pteroylglutamic acid and that the utilization of pantothenic acid and riboflavine should be studied. Meyer has treated 125 patients with acute leukemia with folic acid antagonists. Pteroyltriglutamic acid has not been observed to cause ill effects and has even been observed to cause a remission in the leukemic process in some patients. However, it was the general consensus of opinion that the administration of pteroylglutamic acid aggravates the course of acute leukemia.

PANEL ON HEAD-AND-NECK TUMORS

Moderators, DANELY P. SLAUGHTER, M.D.
University of Chicago, Chicago, Illinois
OLIVER COPE, M.D.
Harvard University Medical School, Boston, Massachusetts
Recorders, ERWIN ROESER, M.D., AND SELWYN TAYLOR, M.D.

In a recent study of the incidence of carcinoma made by Miss Eleanor MacDonald in the State of Connecticut, it was found that the incidence of cancer in the head and neck area constituted 15 per cent of all cancers. Oral cancers alone constituted 5.8 per cent of the total studied. Cervical metastasis were noted to be present in 36 per cent at the first examination of patients having head and neck cancer.

PRECANCEROUS LESIONS

In considering the precancerous lesions, Sunderland pointed out that in the oral cavity, long standing chronic inflammation, keratosis, and leukoplakia were the commonly accepted premalignant lesions. The thin, filmy type of leukoplakia was considered less significant than the thick and hard or the fissuring type. He quoted Sturgis and Lund as reporting that 12 per cent of patients with leukoplakia subsequently developed intraoral cancer. Ill-fitting dentures have not been found statistically to be a contributing cause of carcinoma. However, intraoral cancers were more commonly found in people with a smoking habit. No carcinogenic substance has been demonstrated in tobacco, but tobacco smoke caused mucosal irritation and in some instances a development of leukoplakia. In those patients with carcinoma of the tongue, an incidence of syphilis was found in 30 per cent of the cases. Many had revealed a previous or associated glossitis or stomatitis. He said Ackerman had reported that a large proportion of patients with the verrucous type of carcinoma were in the habit of chewing tobacco. He felt that nutritional deficiencies also might be significant in the production of precancerous lesions. Particularly cited

was the frequent relationship of vitamin-B deficiency with edema, fissuring, and papillary atrophy of the tongue.

Slaughter revealed that once an intraoral carcinoma had occurred in a patient, there was a great likelihood that a new carcinoma might develop in the surrounding mucosa. In a review of eighty patients with intraoral cancer, 18 per cent had or subsequently developed a new carcinoma in the surrounding mucosa and supported the theory of multicentric origin of cancer. In this respect, he pointed out that the intraoral epithelium may be preconditioned to the development of cancer by a different rate of maturation of the epithelial cells. The sudden appearance of a carcinoma near or adjacent to a previously treated lesion need not necessarily be a recurrence. Microscopic slides were shown revealing the development of carcinoma independently in several areas of a cut section. Sometimes they were covered by a normal overlying epithelium.

Schour suggested that a number of prophylactic measures might be taken to prevent the occurrence of malignant or premalignant lesions. Foremost were the correction of malocclusion, pyorrhea, infection, and sharp or irritating teeth. He also urged that excessive smoking and the use of strong mouth washes be discontinued; the latter destroy the normal protection of the mucosa by the saliva. The detection and treatment of syphilis and the replacement of absent teeth, which might also cause malocclusion, was also to be encouraged. The habit of holding foreign objects, such as nails or screws, in the mouth should be discouraged.

Lund felt that the development of leukoplakia probably represented changes produced by one or more weak causative agents over long periods of time. In many instances, this might represent at least a twenty- or thirty-year period of stimulation. He is particularly interested in the fact that leukoplakia in other areas, such as the vulva, appears to be a process very similar to that in the mouth and cites one case in which the administration of stilbestrol caused a disappearance of the leukoplakia of the vulva. He cited two other cases of oral leukoplakia that showed regression and disappearance upon the administration of the estrogen. Weisberger and Nathanson showed that in 42 per cent of thirty-eight patients with oral leukoplakia, complete regression was observed

with the administration of stilbestrol. Lund stated that the reversal of hyperkeratosis to a normal mucosa in his cases was a very slow process. In treatment, surgery and stilbestrol are most important; vitamins and removal of irritants are valuable supplements.

Bernier suggested that the word "leukoplakia" was used in the medical and dental professions in a very loose manner and frequently connoted a hysterical fear of cancer in both the patient and physician. He suggested that "leukoplakia" be reserved for that epithelial change manifested by frank dyskeratosis and stated that this histological change cannot be detected by gross clinical examination. He observed that smoking did not appear to have been a significant factor in 850 cases of carcinoma of the lip. He likewise felt that syphilis, tobacco, and infected teeth should not be considered a direct factor in the development of cancerous and precancerous lesions as had been formerly believed. He felt that these factors contributed only in a general way to neoplastic changes, but that tobacco was probably a more significant factor in carcinoma of the tongue.

Cutler stated that leukoplakia is a physiological keratinization of the mucosa arising from physical trauma. He compared the development of hyperkeratinization of the oral mucosa to calluses. Hence, leukoplakia may well represent a form of protection of the body against injury to the mucous membranes. Advanced leukoplakia is sometimes difficult to differentiate from carcinoma.

Weisberger pointed out that a proper metabolic study of the transition of normal epithelium to leukoplakia has not yet been made and might well reveal some interesting facts about precancerous lesions. He stated that the oral mucosa was ever resisting salivary and food enzymes. To a certain degree, he has been able to detect the advancement of leukoplakia by staining a suitable biopsy for its glycogen content.

Intraoral Cancer

The prognosis in cancer of the tongue might be more accurately predicted, Blady stated, if more critical histological criteria were established. Foremost to be considered were the desmoplastic reaction, degree of mitosis, and basic stroma of the tumor. Mention was made by Nelson that the average patient with carcinoma

of the tongue came to the qualified physician far too late. In his own practice more than 40 per cent had cervical metastases at the time of their initial examination, and another 40 per cent manifested metastases within a very short time.

Windeyer was of the opinion that carcinoma of the anterior two thirds of the tongue were best treated by radium needles, except when the tumor was unusually large and bulky, had invaded the floor of the mouth, or was recurrent from previous irradiation. He had observed only a slight morbidity subsequent to lesions treated in this manner. He felt that the lack of careful planning and homogeneity of radium needles and/or overdosage of radiation is the cause for extended morbidity in some cases. He has obtained a 45 per cent five-year survival in those cases free of cervical metastases when first treated. On the basis of the multicentric origin of carcinoma of the mouth, Sharp formulated the basis of his treatment for carcinoma of the tongue. This consists primarily of administering approximately one third of the tumor radiation through lateral ports and inserting radium needles into the tumor for the remaining calculated dosage. He has discarded the use of the intraoral cone in roentgen-ray therapy.

Kerr pointed out that a very important part of the treatment of oral cancer by irradiation was proper preparation of the mouth. All infection should be eliminated and all of the teeth should be extracted to prevent irradiation necrosis, which is uncontrollable and often responsible for the death of the patient. The removal of all teeth made irradiation a more successful treatment.

Robinson enlarged on this by pointing out that a severe form of uncontrollable dental caries results because of the reduced flow of saliva produced by irradiation of the salivary glands. This occurs when extractions are contraindicated.

Cade stated that the morbidity and postirradiation effects accompanying the treatment of intraoral tumors were almost always due to an overdose of radiation. In his experience, the use of low-potential radium needles in carcinomas of the tongue has resulted in a very minimal morbidity. He insisted that there must first be an edentulous mouth and correction of associated ill states of health before treatment is instituted. He felt that a mixture of

cautery, surgery, radon, and roentgen rays was ill-advised. He called attention to the fact that many of the lesions that are very radiosensitive and disappeared quickly frequently returned in a very short time. He stressed the difficulty of obtaining a tumoricidal dose of roentgen-ray therapy without extensive injury to adjacent normal tissue in those cases with cervical metastases. For this particular problem, he has designed a low-potential radium needle and is able to administer a calculated dosage of 10,000 r.

Binkley proposed that the term "five-year 'cure' " should be replaced by the term "five-year 'control' " since it is misleading to state or report that a patient is cured when as a matter of fact the disease is only under temporary control and may become active again—between the fifth and tenth years or later in life. He emphasized the need for a more critical review of our treatment failures particularly in the early small growths to learn if disorders of metabolism or inherent factors may be at fault. The chemical balance of the body and the patient's inherent physiology were suggested as important factors in the ultimate control of the cancer.

Robinson pointed out that the discussion had centered about attempts of radiation therapists and surgeons to salvage failures in cancer control, i.e., advanced lesions. The Panel, for all practical purposes, omitted discussion of the early recognition of oral cancer in which the dentist must play the major role. The dentist sees the mouths of the patients more often than anyone else and should be adequately trained in the diagnosis of all oral lesions including those of cancer. Discovery of oral cancer when it is still very well localized is most important and offers great hope for prevention of cancer deaths. Investigations by oral pathologists include the development of early methods of diagnosis by such methods as study of the exfoliative cytology of the mouth.

Cervical Metastases

In a study of 144 cases by Charles Martin, a five-year survival of 21 per cent was obtained when radiation was used. He held that external irradiation was of little or no value except in those

instances of node metastasis from a primary transitional-cell carcinoma, and that the Coutard method of administration was the one of choice when external irradiation alone was employed. He preferred the combination of interstitial and external irradiation for the treatment of cervical-node metastasis. This also took advantage of limiting the ill effects from either the beta or gamma rays alone. The tissue irradiation was secured from a combination of these rays. For interstitial irradiation he preferred low-intensity radium needles to radon seeds. The needles were implanted not only in the clinically discerned node, but also into the immediately adjacent tissue. However, he believed that an adequate tumoricidal dose was not achieved from the needles alone and accordingly supplemented irradiation to the area of treatment with 2100 r in the form of external roentgen rays. In most instances, nodes were not biopsied before treatment, but he cites seven histologically proved cases with a five-year survival.

Daland suggested that the treatment of choice be selected as that best suited to the individual case. He believed that radical neck dissection was indicated in those cases in which a combined excision of a primary tumor and its metastases could be obtained. The radical neck dissection could also be employed for either palliation or cure when the primary cancer was controlled. He has performed radical neck dissections through recently irradiated tissue without seriously retarded wound repair. The over-all five-year–survival rate was between 25 and 30 per cent.

Cade expressed the opinion that there was more uniformity in the treatment of cervical-node metastases. He believed that the procedure of choice was radical neck dissection, but only after the primary lesion had been controlled and when the patient's general health could withstand the procedure.

Often cervical metastases grow rapidly during the time of treatment necessary to sterilize or control a primary lesion. If untreated, the diseased nodes may affix themselves to important vascular structures thus rendering subsequent forms of therapy either hazardous or impossible. Slaughter mentioned that he has had the occasion to implant radon seeds into these clinically metastatic nodes to "hold them in check." In this manner, treatment

to the primary tumor could be completed and the metastases prevented from becoming inoperable. Mention was made of the value of prophylactic radical neck dissections. Factors, such as a patient living so far from the source of therapy that frequent examinations were impossible, were cited as an indication for prophylactic radical neck dissection in selected cases. Haven stated that metastatic nodes were found in 10 per cent of the prophylactic neck dissections performed at the Mayo Clinic.

The cases of recurrent intraoral carcinoma, following irradiation, with and without cervical-node involvement, were next considered. Previous to 1942, Catlin stated that the only form of therapy known was further irradiation and that the five-year–survival figure was less than 7 per cent of cases treated. These cases had previously been considered inoperable. At the Memorial Hospital, an en bloc dissection of the primary site of the recurrent tumor with an adjacent radical neck dissection was first performed in 1942. Usually the mandible, diseased or otherwise, was excised in the resection. The necessity of establishing a tracheostomy was shown in the drop of the postoperative mortality from 16 per cent in 1943 to 2.7 per cent in 1947. Reconstruction of the defect from the loss of the mandible was attempted by Catlin in seven cases. The excised mandible was immediately autoclaved and wired back in the proper place as a sterile foreign body. He stated that only one was really satisfactory but that better results were sure to be obtained from a more careful selection of cases.

Modlin cited forty-nine cases of the combined excision of primary intraoral tumor and radical neck dissection. He re-emphasized the necessity of establishing a tracheostomy for this procedure and thought it was especially suitable for tumors fixed to the mandible, whether or not there were cervical metastases. Slaughter revealed that floor of the mouth and gingival lesions also frequently required mandibulectomy as the only way to remove lymphatics in continuity when excision of the primary tumor and its metastases was considered. An alternate procedure of swinging the mandible out laterally to make possible adequate exposure for en bloc dissection in selected intraoral lesions was suggested. Replacement of the autoclaved mandible was employed

by Klopp with encouraging results in three cases. Slaughter suggested that an earlier indication for the combined procedure might be the answer to the failures in this group. As this operative procedure is only a recent concept the ultimate survival rate cannot as yet be evaluated.

Summary

Three main points for future investigation seem to have been outlined. First, intensive investigation of precancerous changes and all epithelial reactions that might lead to cancer must be undertaken. These would include carcinogenic exposure and general metabolic relationships, and local problems of dental and salivary reaction. Second, emphasis should be placed on failure of treatment and study made of these cases, rather than on statistical studies of success. Third, there should be an evaluation of radical and combined surgical procedures for advanced cancer in their application to earlier cases.

Carcinoma of the Larynx

Discussion of carcinoma of the larynx was opened by Cutler who stated that the life expectancy of untreated patients with carcinoma of the larynx was from six months to two years. He pointed out that far too many tumors of the larynx came to the qualified therapist in a very late stage of the disease. In his own experience, he grouped 80 per cent of patients with carcinoma of the larynx in this category. He noted that 95 per cent of patients with carcinoma of the larynx manifested hoarseness as an early symptom. He also stated that carcinoma in this region has steadily manifested a progressive yearly increase. From his study, the average delay of the patient before definitive treatment was instituted was found to be about fifteen months. In selected patients with an early carcinoma of the larynx he stated that the prognosis of a five-year survival when treated by roentgen rays as a suitable form of therapy was about 80 per cent.

Lenz held that the problems of treatment by irradiation were two. Namely, the local extent and character of the disease and the contiguous cartilaginous infiltration. He felt that the histologi-

cal grading of tumors of the larynx was of secondary importance. An advantage of surgical excision as a form of treatment was that it was essentially over in one-week's time. Irradiation required an extended time for the completion of the treatment and often manifested permanent and/or long-lasting general and local reactions. He felt that as a final result, phonation or voice was about the same whether a laryngofissure excision or irradiation was employed. Extensive lesions of the false cords, arytenoid cartilage, or lateral wall of the larynx was best treated by surgical excision. In nineteen cases that fell in this group, four were living seven to fifteen years later. Carcinoma of the epiglottis held a slightly better prognosis with nine of twenty-seven cases being well and free of disease five or more years later. In general, in those carcinomas in which there was considerable fixation of adjacent structures, the prognosis was much less encouraging. Primary carcinoma of the pyriform sinus was most discouraging in that none of twenty-six cases survived. Complications of chondritis and chondronecrosis were mentioned in those carcinomas of the larynx that invaded either the epiglottis or arytenoid cartilages and were treated with irradiation as a primary form of treatment.

In spite of both lay and medical education, it was felt by Frazell that carcinomas of the larynx, by and large, were submitted to formal treatment late in the disease process in far too many instances. This was especially true in cases treated at the Memorial Hospital, many of which had had numerous previous and inadequate forms of treatment. In a review of 187 cases of carcinoma of the larynx that were primarily treated by irradiation, there was survival for five or more years in only 10 per cent of those with extrinsic lesions and in 31 per cent with intrinsic lesions. In contrast, when total laryngectomy was performed for intrinsic lesions, an over-all five-year cure of 58 per cent was obtained. In those cases in which the lesion was suitable to partial laryngectomy, a five-year cure of 57 per cent was found. A most encouraging five-year cure of 95 per cent was observed when the intrinsic lesion was less than 1 cm. in size. In a series of 258 cases in which total laryngectomy was performed, there was an operative mortality of 2 per cent. In

sixty of these cases in which there had been a lapse of five or more years, 62 per cent were well and free of disease.

Clark stated that lesions involving the anterior commissure were best suited to laryngectomy and not the laryngofissure type of procedure. In these instances, he also performed a prophylactic radical neck dissection in continuity with the primary lesions. He likewise advocated an en bloc dissection of epiglottic lesions believing this would undoubtedly give a better survival rate. He stated that lesions of the epiglottis were late to metastasize and usually remained local. It was his feeling that too often emphasis is placed upon the preservation of voice or on economic factors, which led to the relaxation of criteria for the form of therapy best indicated for the disease process. The value placed on life should certainly be more than that of occupation.

In conclusion, it was felt by the Panel that the prognosis of early carcinoma of the larynx was good. The percentage of five-year survivals following either modality of treatment considered might feasibly be increased if there were a freer exchange of criteria and indications among the therapists so that the best form of therapy was always instituted.

Cancer of the Thyroid

The Panel on cancer of the thyroid opened with a review of the incidence and natural history of the various types of carcinoma and a discussion of the established methods of treatment by surgery and external irradiation. In the second half, the functional activity of the carcinomas and their metastases as judged by tracer studies with radioactive iodine I^{131} was presented; the alteration of this activity induced by endocrine change and thiouracil was also discussed. The Panel closed with a presentation of experimental tumor formation and the possibilities for future investigations.

The *incidence* of thyroid cancers encountered in the Chicago area was presented by Slaughter. During the period 1936 to 1948, there were 1.4 per cent of cancers in 290 multinodular toxic goiters. Among 285 nontoxic nodular goiters, the incidence of carcinoma was 17.2 per cent, 9.8 per cent occurring in multinodu-

lar glands and 24.4 per cent in solitary nodules. Dobyns gave the figures for the Massachusetts General Hospital during the twelve-year period, 1937 to 1948 inclusive. There were 1328 benign nodular goiters and 105 primary carcinomas operated upon; 7 per cent of nodular goiters and 19 per cent of single nodules proved to be malignant. Pflueger of San Francisco presented figures which were in general agreement with those from the East Coast and stressed the high incidence of cancer occurring in goiter in childhood. Duffy stated that at the Memorial Hospital there had been thirty cases of thyroid cancer in children. In the subsequent discussion, it was made clear that the cases seen at the Memorial Hospital were highly selected and that these figures did not offer a true picture of the incidence of thyroid cancer in childhood.

Crile presented a critical survey of hospital statistics on the incidence of cancer. He stressed that in a goiter district such as Cleveland, Ohio, 5 per cent of all patients passing through the clinic had nodules in their thyroids, and that the incidence of carcinoma was so low in those having nodules that prophylactic thyroidectomy for nodules was contraindicated. Statistically, he postulated that prophylactic mastectomy for breast cancer would be a more rational use of surgery. He concluded that the decision for operation on a nodular goiter should be based on clinical findings suggesting malignancy.

The *natural history* of the various types of cancer was described by Frazell on the basis of 210 cases seen at the Memorial Hospital from 1935 to 1945:

Papillary	49%
Follicular and alveolar	8%
Hürthle-cell	9%
Giant-cell	15%

Patients free from tumor five years or more after treatment were, by histological type:

All types	30%
Papillary	50%
Follicular and alveolar	30%
Hürthle-cell	30%
Solid	15%
Giant-cell	0%

The papillary type comprised about one half, which was fortu-

nate, since they were the least malignant. They were mainly confined to one lobe, but about 25 per cent involved the isthmus and the opposite lobe. About 20 per cent extended beyond the confines of the gland to invade neighboring structures, but extensive treatment by surgery and irradiation had been encouraging. Metastasis from this type of thyroid cancer was characteristic, tending to involve the jugular lymph nodes, although the primary tumor might be minute. Follicular and alveolar tumors made up only a small group, the chief feature being an attempt at reproduction of the thyroid follicles. Metastases frequently occurred in bone, but their appearance might be long delayed. Hürthle-cell tumors, which comprised a specific histological cell type, might simulate a solitary adenoma and metastasize to lung, bone, and cervical lymph nodes, in that order. Solid thyroid tumors form the second most common type. Their microscopic pattern is pleomorphic, and they run a characteristic, highly malignant course. Giant-cell tumors often have a spindle-cell component. Such tumors are often notably large, involve more than one lobe, are highly infiltrative, and invade neighboring structures. They are usually inoperable when first seen, metastases to nodes and lungs occur more often, and death is frequently caused by rapid tracheal obstruction. Such cases rarely survive longer than one year.

Frantz, of New York, described how she differentiated between these tumors when teaching medical students. She grouped them into three types: good, bad, and those whose prognosis could not be predicted. She stressed that every transition was seen between different histological types and that the pathology often defeated both pathologist and surgeon. Sunderland also supported this view that no clear-cut dividing line could be drawn between different types.

The functional capacity of various tumors, that is, the evidence for production of the thyroid hormone by malignant tumors, was described by Frantz by illustrative case histories of patients who had received I^{131}. Schmeisser presented for discussion the problem of a patient with a Hürthle-cell carcinoma with metastasis. Slaughter knew of such a case treated by Dr. Dwight Clark of Chicago, in which metastasis had shown a significant I^{131} uptake;

the patient had previously had a radical neck dissection with, presumably, a total thyroidectomy. In reply to the question of Chamberlain as to whether the increased avidity for iodine of the metastases following total thyroidectomy was due to the lack of competition for iodine, it was pointed out that such an operation caused an endocrine change in the body, which presumably resulted in increased anterior-pituitary activity and possibly hormone stimulation of the secondary deposits.

Treatment

Crile advocated nonmutilating operations, multiple if necessary, for the sluggish papillary tumors of low malignancy in young women. He stressed the importance of superior mediastinal invasion. He recommended radical extirpation, including the sternomastoid muscles for the more malignant adenocarcinomas, and heavy interstitial irradiation for inoperable lesions. Taylor of Boston was opposed to picking the "individual blueberries out of the muffin" and advocated the radical operation, including the removal of the sternomastoid muscles, in all cases with lymph-node involvement.

Treatment by *external irradiation* was first discussed by Sherwood Moore. Phillips classified the lesions treated by roentgen rays into three groups.

1. Carcinoma Simplex: This term included those very malignant tumors with a short natural history, which were usually inoperable when first seen but very responsive to irradiation. The treatment given was 4000 r in four weeks, but few cures resulted, and the patient usually died of metastases involving liver and lung, rarely bone and brain. Palliative treatment with 2000 r given over ten days was worth while; by this means, hemoptysis could be stopped and solitary lung deposits controlled. It was useless to irradiate when multiple secondaries were present.

2. Malignant Adenoma: This term implied a tumor that had metastasized. Usually there was not need for postoperative irradiation, since the spread was rarely confined to the neck. Preoperative irradiation, however, could be useful, especially if 2000 r was given over ten days and the operation performed on the eleventh. Sur-

vival for five years or longer had occurred in 40 per cent, but these patients were not cured and when metastases occurred they had to be irradiated.

3. Papillary Adenocarcinoma: This term included those tumors occurring before the age of 30 years, which spread typically to the local nodes. Late dissemination was a danger that could be prevented in 75 per cent by postoperative irradiation to judge by the five-year–survival rates; 3000 r in three weeks should be given to the lower two thirds of the neck and the superior mediastinum; however, late secondaries still occur. Windeyer gave his results in a smaller series of cases than the previous speaker and said that he was not so pessimistic as some workers in this field. He had found the undifferentiated tumors very radiosensitive and had irradiated them widely. This had resulted in satisfactory palliation for two or three years. In giving postoperative therapy for the papillary type of tumor, he employed a filtered hard beam or, alternatively, a radium beam, for those areas where it was possible that the cancer tissue had not been completely removed.

The *isotope therapy* of thyroid cancer was introduced by Trunnell (New York) with a brief description of the properties of I^{131} and the methods by which it might be used. The adequate irradiation of tumor tissue depended on its iodine uptake and this could best be shown by radioautographs. He therefore proceeded to outline the findings in the various histological types using this technique. Of twenty-five papillary carcinomas, eight were of the so-called "pure" type and none of these took up I^{131}. All the remaining seventeen mixed papillary tumors had some degree of I^{131} uptake. Of thirty-three mixed adenocarcinomas, twenty-four showed blackening of the radioautograph film while nine showed none. Amongst eight solid adenocarcinomas, four were mixed in type and took up I^{131}. Four were of the "pure" variety, and only one of these gave a positive radioautograph. Of seven Hürthle-cell tumors, only one took up I^{131}. Five giant-cell and three anaplastic tumors had no uptake. Thus, of the total eighty-nine tumors studied, thirty-nine showed significant uptake of I^{131} by radioautograph. A small number of patients with malignant thyroid disease had been treated with therapeutic doses of I^{131}; amongst

these two had definite prolongation of life with regression of the tumor. There have been no cures.

The problem which next came under discussion was how to increase the uptake of iodine by the secondary deposits. The first line of attack was total thyroidectomy carried out surgically (or with I^{131}). Of twenty-four so treated, twelve showed an increased I^{131} uptake in the metastases; this was sufficient in some to make treatment possible. The histological appearance of the primary tumor was not considered important, since the metastases differed in appearance not only from the primary, but also from each other. Thyrotropic hormone was found in seven cases to increase the activity of the secondaries. Prolonged thiouracil treatment had also been employed with some success. Finally, all patients were given a low iodine diet in order to make the tumor cells more avid for iodine.

The dangers of I^{131} therapy were also discussed. First, there was damage to all the normal tissues exposed to the irradiation. Bone-marrow activity could be suppressed, especially when such heroic amounts as 315 mc. I^{131} had been given in a single dose. Changes followed in the peripheral blood, and one patient had died of pancytopenia. There had also been a cessation of menstruation in three patients, but in one of these it appears to be returning after nine months. One of the difficulties of obtaining a uniform irradiation of the malignant tissue was that radioautographs showed a spotty uptake, indicating that the iodine distribution was not evenly spread throughout the tumor. Windeyer asked whether divided doses might not give a more consistent uptake, since suppression of a series of different groups of cells might allow more of them to be irradiated. Crile supported this suggestion, since theoretically, as each new batch of cells was irradiated, there would be an increased stimulus to activity on the part of the remaining tumor tissue.

Dobyns presented the results from autoradiographs made on all thyroid tumors removed during the last three years at the Massachusetts General Hospital. He showed typical examples of the spotty uptake and stressed that the histological picture could not always be interpreted with certainty in terms of secretory activity.

He then described a patient with a metastasis in the ilium that had significant I^{131} uptake six months after total thyroidectomy. A biopsy was taken, and the tumor stimulated by thyrotropic hormone, following which a further biopsy showed a loss of colloid with vacuolation. The nuclei became larger, vesicular, and rose to a position well above the base of the cell. These changes were accompanied by a good uptake of I^{131}, which was administered in therapeutic dosage. Frantz referred to Marinelli who had the good fortune to investigate a patient with multiple bone secondaries that were hyperfunctioning as shown by the elevated basal metabolic rate, response to Lugol's solution, and to thiouracil. Such tissue takes up the largest amounts of iodine known, yet it was found impossible to give a curative dose of I^{131}. However, the disease has been held in check by repeated I^{131} treatment.

CARCINOMAS AT THE MASSACHUSETTS GENERAL HOSPITAL
JANUARY, 1937, TO DECEMBER, 1948

Benign nodular goiters	1328
Carcinoma in nodular goiter	100
Carcinoma in diffuse hyperplasia	5

7.0% of nodular goiters contained carcinoma
19% of 156 single nodules were carcinomatous

TYPES OF CARCINOMA

	No.	*Av. Age*	*Youngest Oldest*
Papillary	54	45.6	16-76
Alveolar adenocarcinoma	21	48.0	17-62
Undifferentiated	25	58.3	32-80
Epidermoid	2	61.5	56-67
Lymphoma	1	65.0	....
Reticulum-cell sarcoma	2	65.0	55-76
Secondary	11	55.6	39-74
Total	116		

PREOPERATIVE CLINICAL FINDINGS IN THYROID*

	Diffuse	*Multi.*	*Sing.*
Papillary adenocarcinoma	9	8	30*
Alveolar adenocarcinoma	0	4	12*
Undifferentiated	9	2	13
Sarcoma-Lymphoma	3	0	0
Epidermoid	1	0	1
Total	22	14	56

* Exclusive of cases previously operated upon, about which facts are not known.

WAS CARCINOMA SUSPECTED BEFORE SURGERY

	Suspected Ca.		*Pos. Nodes*
	No	*Yes*	*Clin.*
Papillary	20	31	26
Adenocarcinoma	10	8	7
Undifferentiated	4	20	6
Sarcoma-Lymphoma	1	1	2
Epidermoid	0	2	3
Total	35	62	44

METASTASES

	No. Metas.	*Pos. Metastases*			
		Lym. Nodes	*Bone*	*Lung*	*Others*
Papillary	18	32*	3	1	3
Adenocarcinoma	8	7	5*	2	1
Undifferentiated	7	10	1	7*	3
Epidermoid	—	2	—	—	—
Sarcoma-Lymphoma	—	3	—	—	—

* Exclusive of cases previously operated upon, about which facts are not known.

ETIOLOGICAL FACTORS AND EXPERIMENTAL CANCER

These were described by Money who had been working on white rats at the Massachusetts General and Memorial Hospitals. These animals rarely developed spontaneous thyroid tumors, hence an attempt was made to induce tumor formation by the prolonged administration of thiouracil. Under such conditions, the gland was presumably subjected to thyrotropic hormone for a long time and remarkable histological changes were induced. Cells lining the thyroid follicles became heaped up, eventually almost filling the whole follicle. The cells then underwent a surprising change becoming tall and arranging themselves as if surrounding new follicles. Occasionally a change occurred to a cell picture which appeared under the microscope to be malignant. Tumors resembling most of those seen in human thyroid cancer were reproduced. Such areas would not grow when transplanted into the anterior chamber of guinea-pig eyes. When the periodic injection of a carcinogen, in this case dibenzanthracene, was added to the thiouracil treatment, they had, however, survived transplantation into the groin of a rat.

Slaughter and Crile then both recounted those thyroid cancers that they had either seen arise or heard had arisen in thiouracil-

treated patients. Dobyns pointed out that in Money's experiments, thiouracil had been administered for a much greater proportion of the life span of the rat than was employed in man.

Possibilities for Future Investigation

In conclusion, Rawson outlined possible future investigations. In a most stimulating talk, he described how the new tools in thyroid investigation were providing new facts. He suggested that now that labeled tyrosine was available, and especially if it could be provided with C^{14} in the benzene ring, it would provide a great deal of new information.

Since manganese has been shown to be picked up by the thyroid, studies with radioactive manganese should be attempted. P^{32} and labeled thiouracil could also be used in man. The effect of estrogens and androgens and pregnancy on thyroid cancer required close study, and two other promising fields for investigation were the application of Astwood's work on the goitrogens in our daily food and the screening of the body's tissues from the unwanted radiation of I^{131} during therapy.

Probably the most important problem that at present requires study is that of determining the mechanism by which the thyroid-stimulating hormone exerts a growth-promoting effect on the normal thyroid cell and on thyroid cancer cells. He also called attention to the fact that thiouracil does not exert the same type of block to iodine utilization in functioning thyroid cancer as in normal thyroid tissue. He suggested that this indicated differences in the enzyme system in utilization of iodine in normal and malignant tissue.

PANEL ON LUNG CANCER

Moderators, ALTON OCHSNER, M.D.
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WALTER E. HESTON, PH.D.
National Cancer Institute, Bethesda, Maryland
Recorder, PAUL T. DECAMP, M.D.

It was generally agreed by the Panel that the differentiation of primary cancer of the lung into the two groups of lung cancer and cancer of the bronchus is unsound, since practically all cancer of the lung is bronchogenic in origin. It was recommended that, for reporting purposes, these two groups be combined and that this differentiation be discontinued as soon as possible by statistical agencies.

INCIDENCE

In the studies at the Charity Hospital of Louisiana in New Orleans and in the St. Louis Municipal Hospital, over five-year periods, bronchogenic carcinoma was found to be more common in men than carcinoma of the stomach. This is at variance with the rates reported in the brochure prepared by the American Cancer Society for the National Cancer Conference. All agreed that the incidence of cancer of the lung is apparently increasing rapidly and that it is one of the most common of human cancers in men.

A wide divergence in reported *sex incidence* was noted, the incidence in men as against that in women varying from 6:1 up to 25:1. No adequate explanation for this discrepancy is apparent. In early reported series, the predominance in men was much less than it has been in more recently reported ones. Also, the absolute incidence is increasing in both sexes, but apparently much more so in the male sex. This may be due to the fact that men are being exposed to a greater number of exogenous carcinogenic factors. No significant difference in the incidence of cancer of the lung has been noted in the admittedly rather inadequate reports compar-

ing the incidence in rural and industrial areas. Careful analysis of the comparative incidence from these areas in relation to the size of the population groups should furnish valuable information.

Incidence by Histological Types. A major problem was recognized in the lack of uniformity in the diagnosis and classification of tumors of the lung, particularly with reference to the so-called

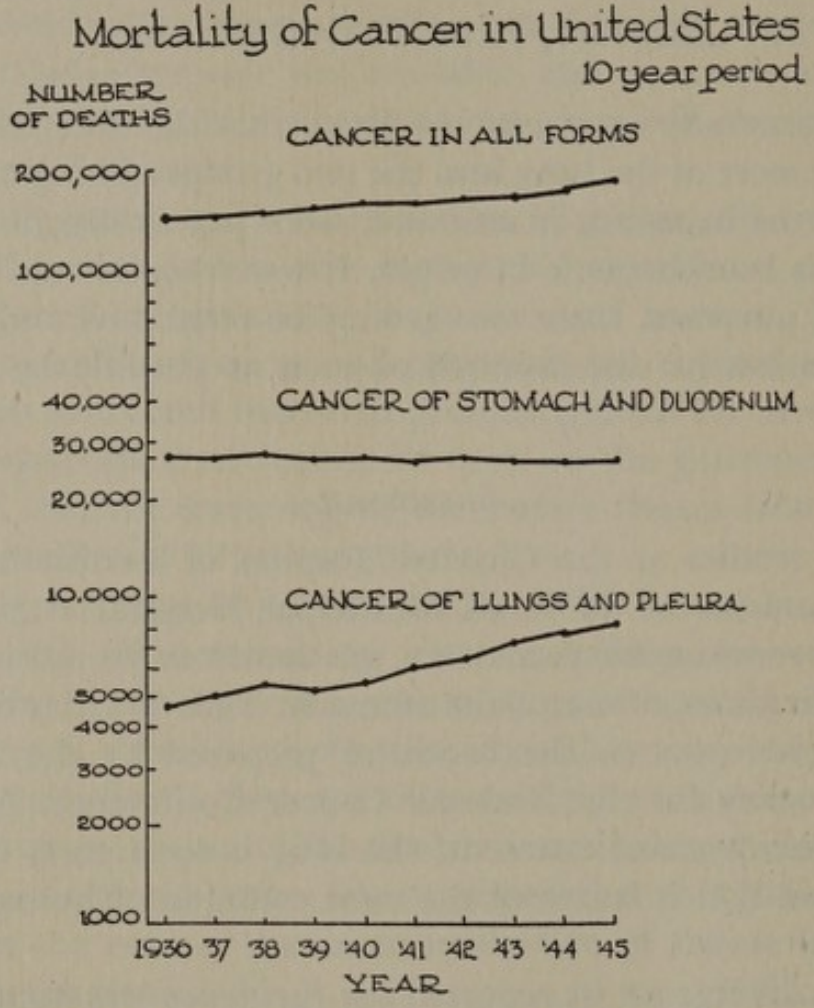

FIG. 1. Mortality of cancer in the United States for a ten-year period.

"adenomas" or "mixed tumors." The inclusion or exclusion of such tumors in a single series made a 350 per cent difference in late results. Nor has there been any general agreement as to how these tumors should be classified. The Panel agreed that they should all be considered as malignant or potentially malignant tumors. It was not felt, however, that other tumors that are definitely benign should be included with definite carcinomas. The circulation of

slides among pathologists was not considered to be a solution of this problem. It was felt that in some instances of established carcinoma, it is impossible to distinguish those which arose from preceding adenomas, either on clinical or on histological grounds. It may be considered the consensus of the Panel that the group of tumors commonly called "mixed tumors" should be included within the classifications of cancers of the lung, but that valuable information may be obtained by considering them as a subgroup with significantly different clinical characteristics.

Diagnostic Procedures

Roentgen-Ray Screening Methods

A few preliminary reports were given on the incidence of asymptomatic cancers of the lung found by universal roentgenographic chest screening methods. The Minneapolis survey of approximately 300,000 individuals revealed 7079 individuals with lesions suggestive of tuberculosis, cancer, or other pulmonary pathology. Recommendation was made that they be examined further by their physician. Of this number, 55 per cent were reported back to the Health Department. Among these cases, six primary carcinomas and nine secondary carcinomas of the lung had been found. An additional twelve carcinomas of the lung were reported but not classified. In twelve additional instances, an absence of the breast was noted together with the presence of metastases in the lung. In twenty-five cases, primary cancers were reported in locations other than the lung and breast. Of forty-five solitary tumors in the lower lobe, twelve were studied and, of these, three proved to be primary bronchogenic carcinomas. It is estimated that if complete returns had been made, ten primary bronchogenic carcinomas would have been found per 100,000 persons studied.

The age distribution of the population screened is very important relative to the number of cases of cancer that will be detected by this means. It was suggested that the value of this method may be increased by selection of appropriate age groups in which the expected incidence would be higher. Such surveys should be encouraged. In addition to the value derived from discovering

early cases of lung cancer, it was felt that such surveys will have very great value in educating radiologists and physicians to a consciousness of early asymptomatic cancer of the lung. It was emphasized that this is the only type of internal cancer that can be detected in a high proportion of cases, in its incipiency, by such a simple screening method.

In a series of 100 cases of silent chest tumors found at the Memorial Hospital in New York, twenty-four proved to be malignant and seventy-six nonmalignant.

Smears of Sputum and Bronchial Washings

The great value of the cytological method of diagnosis of cancer of the lung was recognized by all. It was agreed that diagnosis by bronchoscopic biopsy has been successful in 40 per cent or less of clinical cases and that this group represents largely one in which the disease is advanced.

The method of cytological study is admittedly a difficult one and for best results requires close teamwork. Expert bronchoscopic technique, where bronchoscopic aspiration is used, is essential; and in every instance, careful and usually time-consuming preparation and examination of the specimens in the laboratory by persons with experience in the method of cytological diagnosis are essential for the best results. Under such circumstances, positive diagnoses in from 85 to 90 per cent of cases of carcinoma of the lung are reported, with only a very low incidence of false-positive diagnoses. It is apparent from these figures that, under these circumstances of diagnosis, a positive diagnosis is fairly reliable, although occasional false positives are reported. On the other hand, a negative diagnosis is by no means reliable in excluding the presence of cancer. Unless some other diagnosis can be established, exploration of the thorax is indicated.

When bronchoscopic aspiration, followed by early examination of the aspirated material, or of sputum, is performed in a less satisfactory manner, the method can still be valuable in increasing the incidence of positive diagnoses and of even greater value in stimulating the interest of the medical profession in the early diagnosis of cancer of the lung. It was recognized, however, that under such circumstances more caution must be exercised in interpreting the

diagnoses made. The incidence of positive diagnoses is lower and the occurrence of false-positive diagnoses is undoubtedly greater.

Thoracic exploration must not be postponed because of negative reports in suspected cases. On the other hand, some caution must be exercised in performing primary radical pneumonectomy in doubtful cases. In such instances, preliminary direct biopsy, segmental resection, or lobectomy with immediate tissue examination, may be indicated.

Reports were received indicating that the examination of sputa is an almost equally valuable although perhaps a more difficult method of examination. Most reports were given on the basis of examinations of smears. It was suggested that embedding the material has the advantage of concentrating it, of providing a more uniform histological specimen, and of preserving the continuity of the tissues. In conclusion, the fear was expressed that this admittedly valuable diagnostic method may be the cause of considerable harm and may come into disrepute if it is widely practiced by incompetently trained personnel.

Aspiration

Aspiration of a pulmonary tumor through the intact chest wall was considered to have limited usefulness, and there was disagreement as to the advisability of the procedure. It was felt it could be dangerous, particularly in an emphysematous lung. Aspiration is indicated when inoperable lesions are present but a positive diagnosis is needed, or where grave danger would attend the exploration of a poor-risk patient. It has more value at operation when deep intrapulmonary or intramediastinal tumors are found and direct tissue biopsy is considered inadvisable. Immediate examination of the specimens thus obtained is helpful in determining the further operative procedure.

Therapeutic Test

The therapeutic test of roentgen-ray radiation on intrapulmonary lesions was condemned. Its application to bilateral mediastinal tumors was considered sometimes helpful, provided delay was avoided if the tumor did not respond to the roentgen-ray treatment. The chemotherapeutic tests of intrapulmonary lesions can

be very misleading by causing the clearing of inflammatory lesions associated with a carcinoma. Such clearing may also occur in a patient with a hilar lesion who is bedridden while receiving

TABLE 1
BEHAVIOR OF TYPES OF CARCINOMA OF THE LUNG

		% Bronchogenic carcinoma				% Alveolar-cell carcinoma
		Squamous-cell	*Pleomorphic-cell*	*Undiff.-cell*	*Adenoca. bronchial glands*	
	% Cases	27	9	*42*	22	1, 3
	Av. Age	46.9	*37.7*	45.3	42	*48.3*
Symptoms	Cough	*93*	78	71	59	66
	Pain, chest	*82*	78	76	73	55
	Hemoptysis	33	*44*	36	27	0
	Weakness	26	*56*	36	18	*55*
Duration	6 mos.	37	45	*57*	54	44
	6-12 mos.	30 } 67	*44* } 89	26 } 83	36 } *90*	22 } 66
	1-2 yrs.	*26*	0	14	5	*33*
	2-4 yrs.	7	*11* (8 yrs.?)	2	5	0
Bronchoscopy	% Reported	*70*	11	38	32	56
	% Positive	*66*	0	30	27	0
	% Negative	*4*	11	8	5	*56*
Metastases	Regional lymph nodes	59	*78*	66	59	0
	Lungs	26	44	41	*55*	0
	Liver	19	33	*59*	45	0
	Adrenals	19	*56*	34	55	0
	Brain	15	*44*	34	32	0
	Kidneys	26	22	26	*41*	0
	Bone	26	..	24	*32*	0

roentgen-ray treatment, falsely suggesting response of the tumor to the irradiation.

TREATMENT

Medical Treatment

It was recognized that neither roentgen-ray nor nitrogen-mustard therapy should be considered a satisfactory curative method

of treating cancer of the lung. Some benefit in the palliative treatment of inoperable lesions was reported by both methods. It was suggested that roentgen-ray therapy be used selectively only; it was recommended in cases in which moderate mediastinal obstruction is present. If the mediastinal obstruction is severe, it should not be used, for increasing edema may aggravate the obstruction. Methyl-bis should be used in these circumstances. The Memorial Hospital group reported some good primary results in oat-cell carcinoma by the combined use of roentgen rays and methyl-bis. It is their experience that dramatic early benefit may occur. This is followed in a relatively short time by an uncontrollable recurrence of the disease. The psychological benefit of doing something for the patient was cited. Nitrogen mustard must be recognized, however, as a potentially dangerous form of treatment because of its side effects.

Surgery

The need to base all statistical studies on the total number of patients with proved or with strong presumptive evidence of carcinoma was recognized by all. There was disagreement among panel members as to the indications for inoperability. Some of this disagreement arose from an admittedly fundamental difference of opinion about the wisdom of palliative resections of carcinomas of the lung that are incurable. On the one hand, a conservative group felt that the higher operative mortality rate incident to palliative resections with its effect in reducing the expected longevity of the group contraindicates pneumonectomy in some of the technically resectable but obviously incurable cancers. On the other hand, all cited the progressive decrease in operative mortality rates. In earlier years, this was 50 per cent or more, whereas in recent years, it has dropped to the neighborhood of 10 per cent in the hands of the more radical surgeons and in the neighborhood of 5 per cent for the more conservative. It was felt by the group advocating radical palliative operations that a significant increase in longevity is obtained and that the increase in comfort to the patient incident to the removal of the lung containing the tumor and its attendant sepsis is an even more satisfying result. The re-

sectability rates of the conservative group range between 25 and 30 per cent of cases operated on, whereas the resectability rate of the more radical surgeons range to as high as 59 per cent of all cases seen in one series.

Histological Type of Disease and Cure Rate

An over-all five-year–survival rate of 5 to 7 per cent was reported by two groups. Several groups reported a 40 per cent five-year–survival rate in cases in which pneumonectomy was performed and the disease was found to be limited to the lung. Where pneumonectomy was performed and the disease was found to have extended beyond the lung, five-year–survival rates were reported ranging from 10 per cent to as high as 20 per cent. The survival of a patient for longer than two to three years when no treatment, or any nonsurgical form of treatment, has been applied is extremely unusual.

It was generally agreed that the squamous-cell carcinomas and the adenocarcinomas carry a better prognosis than the undifferentiated-cell types. There was sharp disagreement concerning the prognosis in oat-cell carcinomas. Many observers had found that this type of carcinoma carried an extremely poor prognosis and, in the opinion of some, was a contraindication to radical surgery. It was the experience of others, however, that such tumors carry a better prognosis than do the large-cell variants in the undifferentiated-cell types of carcinoma. These urged attempts at radical surgery for this type of tumor.

Reporting of Lung Cancer

A report was received on a tentative draft of the recommendations of the Panel on Reporting of Clinical Cancer. This group found serious deficiencies in present statistical reports and cited the following needs:

1. There is need for carefully defining of the group of cases included in a given report. It is recommended by the aforementioned panel, and enthusiastically endorsed by the Panel on Lung Cancer, that reports should be based upon all cases seen with the

disease in question. A classification of cancer of the lung from the therapeutic point of view is suggested as follows:

1. Considered inoperable.
2. Considered operable.
    a. Refused surgery.
    b. Exploration of thorax.
    (1) Resected.
    (2) Not resected.

Specification should be made whether microscopic proof had been obtained. Further subdivision of cases is desirable but this minimum classification was recommended by the Panel on Reporting of Clinical Cancer. As a more general classification applicable to all forms of treatment, it suggested the following:

1. Treated for cure.
2. Treated for palliation.
3. Not treated.

2. Many existing reports lack a high percentage of follow-ups. The complete inability to draw any valid conclusions under such circumstances is emphasized.

3. The problem of adjusting for deaths for causes other than cancer was stated. In many instances, the original physician treating the patient may not have contact with him at the time of his death. It was suggested that the survival of patients treated for cancer of the lung be compared with the expected survival of persons in comparable age and sex groups who do not have the disease. If this comparison is used, it is not necessary that the cause of death be known.

The Panel on the Reporting of Clinical Cancer made the following recommendations:

1. That the suggested forms for presentations of five-year end results for diagnosed cases of cancer be distributed to selected individuals for application to present and to past series of cases of cancer. This was suggested in an effort to evaluate the usefulness of these forms in a relatively brief period of time.

2. That the American Cancer Society set up a subcommittee of the Statistics Committee of the American Cancer Society for further study of this problem.
3. That the American Cancer Society provide consulting assistance to clinicians in the statistical analysis and evaluation of their end of diagnosed cases of cancer.
4. That the American Cancer Society should encourage the wider distribution of more detailed data on end results in cancer:
   a. by requesting suitable space in selected journals for articles on this subject;
   b. by acting as a clearing house in reproducing and distributing supplementary data to regularly published articles.

It was stated that the Medical and Scientific Committee of the American Cancer Society had considered these recommendations and felt that as soon as statistics improve, such reports will be more readily accepted by medical journals. The projected function of the American Cancer Society as a clearing house for supplementary data was further explained, and it was suggested that after publishing an article, the author send to the American Cancer Society, on work sheets supplied by that agency, the tabulations upon which the published conclusions were based. It was hoped that, in this way, presently available statistics could be more critically evaluated and serve as a more useful basis for comparison.

Representatives of several public-health departments stated that, in their experience, such departments can render invaluable service in tracing patients who have been under treatment for cancer.

The need for the clarification of nomenclature was reiterated. It was pointed out that the Chest Tumor Registry has already been set up and can be used for that purpose. It was suggested that the American Cancer Society co-operate in this activity.

PROBLEMS IN THE CONTROL OF LUNG CANCER

The present delay in the diagnosis and treatment of cancer of the lung is deplorable. A series of 200 cases was reported in which there was an average of ten months between the first symptom of the disease and the arrival of the patient in the hands of a surgeon

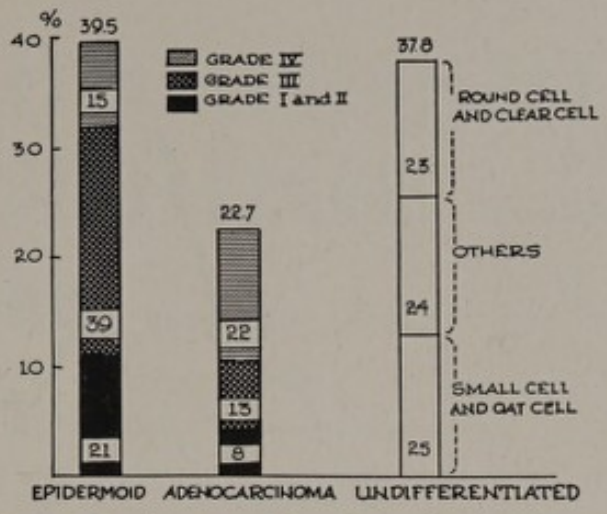

FIG. 2. Carcinoma of the lung; distribution by histological type and grade.

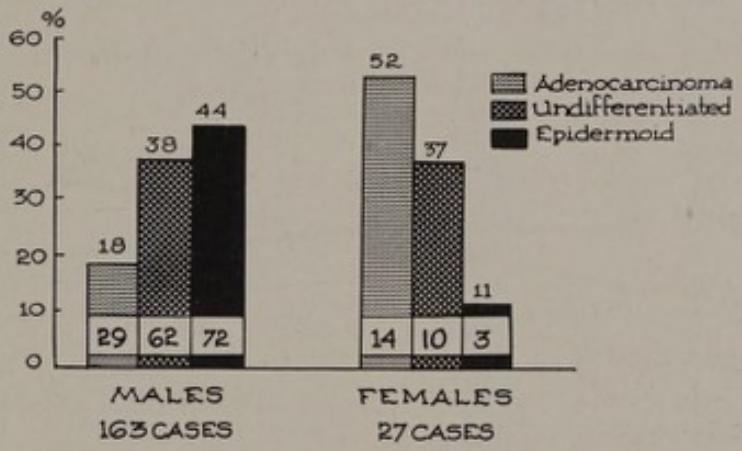

FIG. 3. Carcinoma of the lung; sex distribution by histological types.

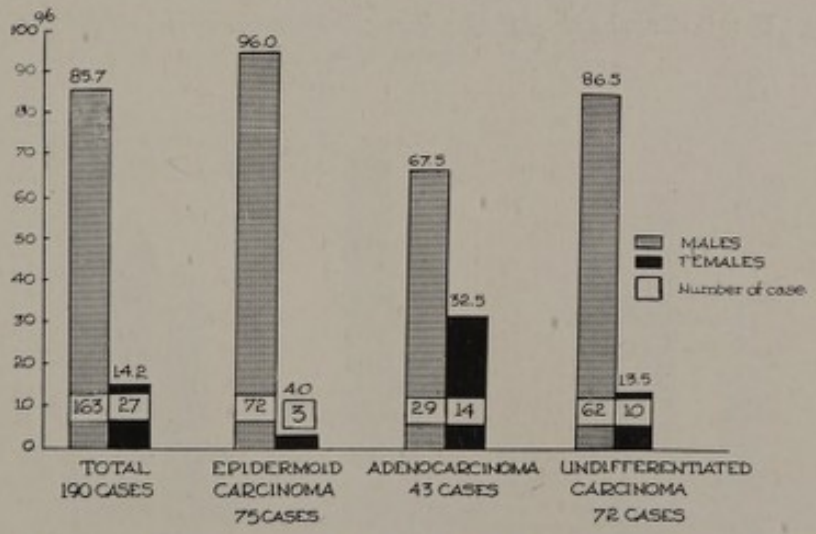

FIG. 4. Carcinoma of the lung; sex distribution by histological types. Figures in squares equal number of cases in column.

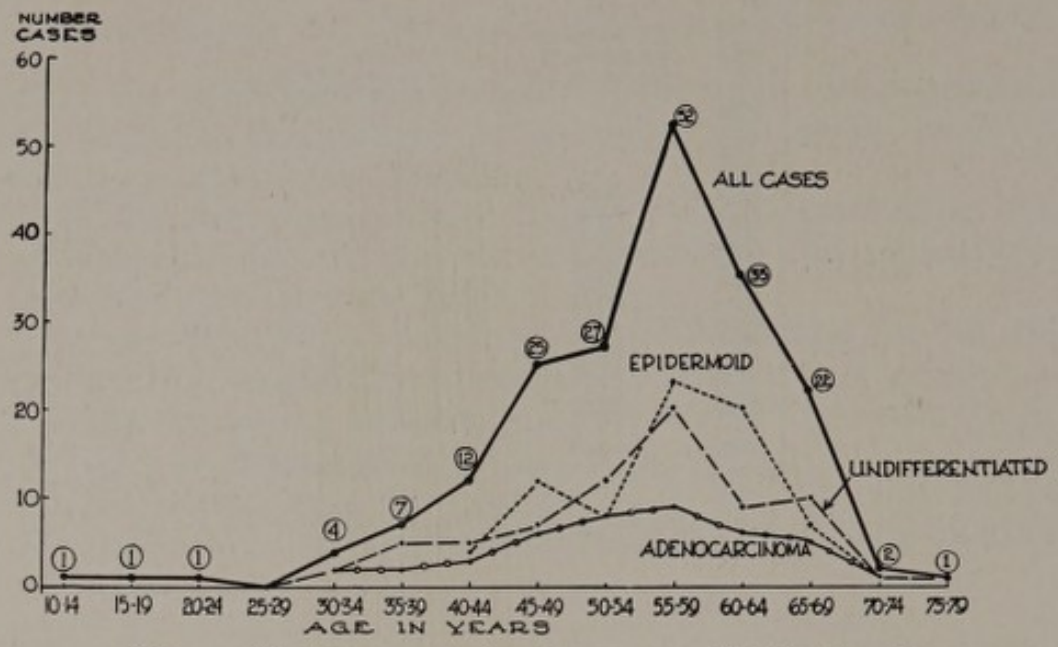

FIG. 5. Carcinoma of the lung; age distribution by histological types.

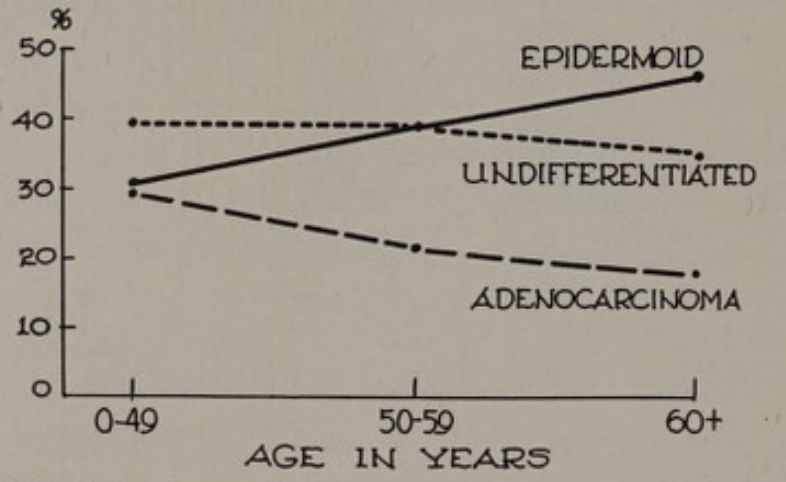

FIG. 6. Carcinoma of the lung; incidence of histological type according to age.

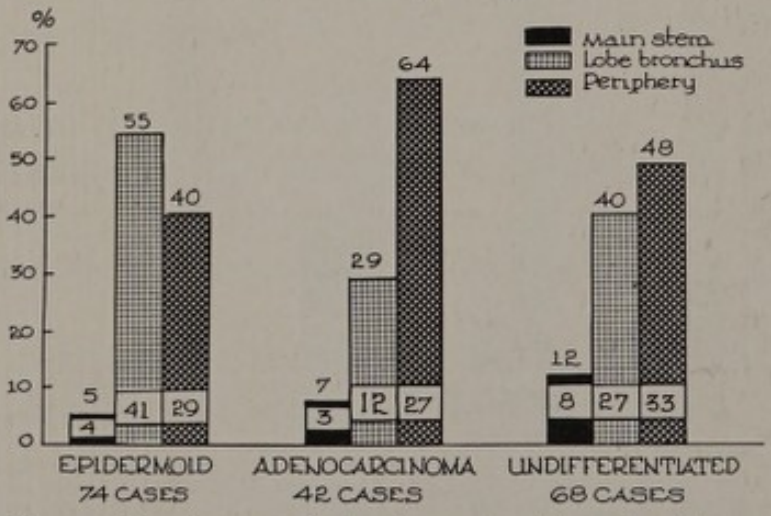

FIG. 7. Carcinoma of the lung; location of primary lesion in the bronchopulmonary tree.

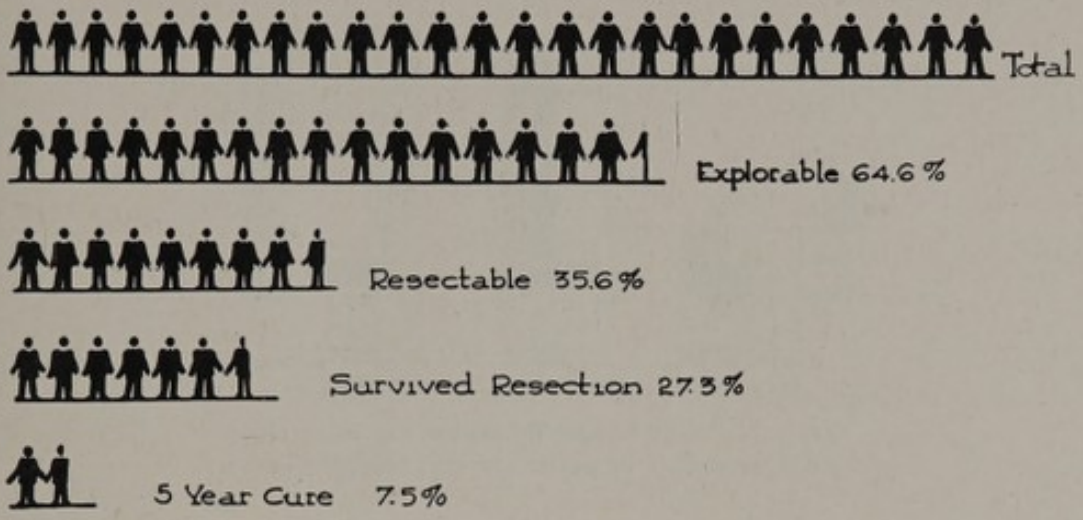

FIG. 8. Carcinoma of the lung; management and results in 548 cases.

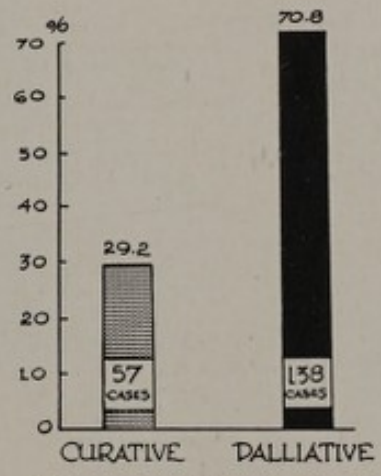

FIG. 9. Primary carcinoma of the lung; incidence of type of resection.

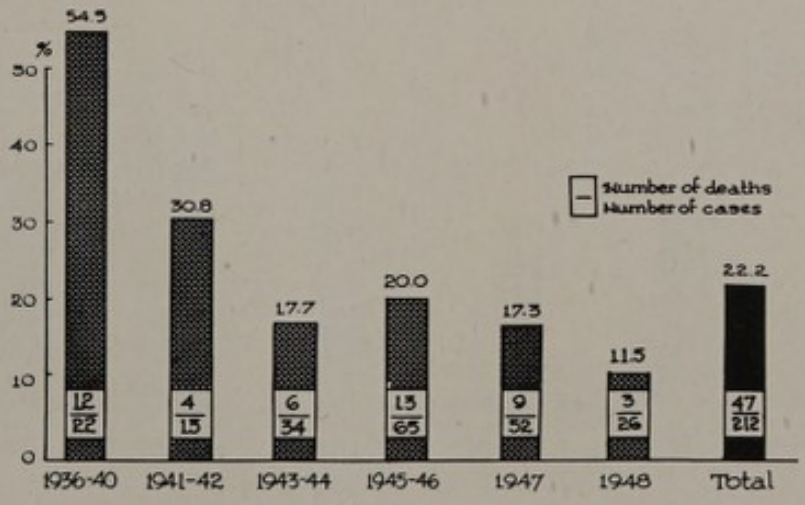

FIG. 10. Carcinoma of the lung treated by resection; hospital mortality in 212 cases.

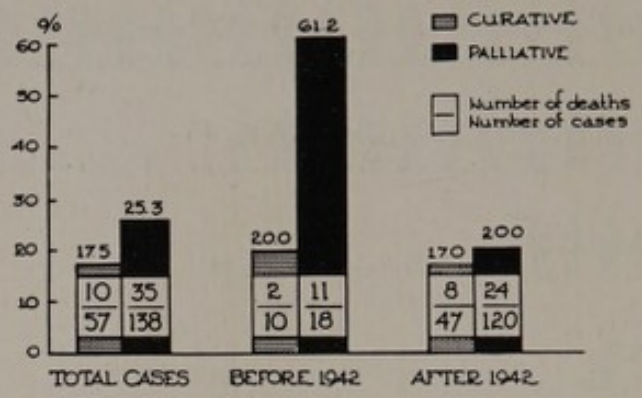

FIG. 11. Primary carcinoma of the lung; hospital mortality in palliative and curative cases.

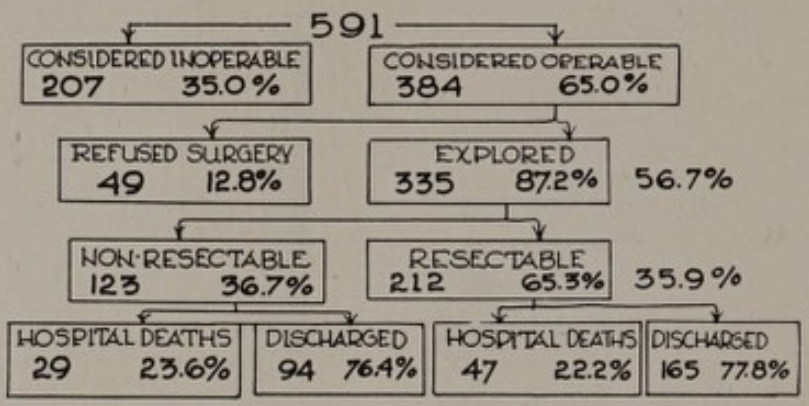

FIG. 12. Results in 591 cases of carcinoma of the lung diagnosed clinically.

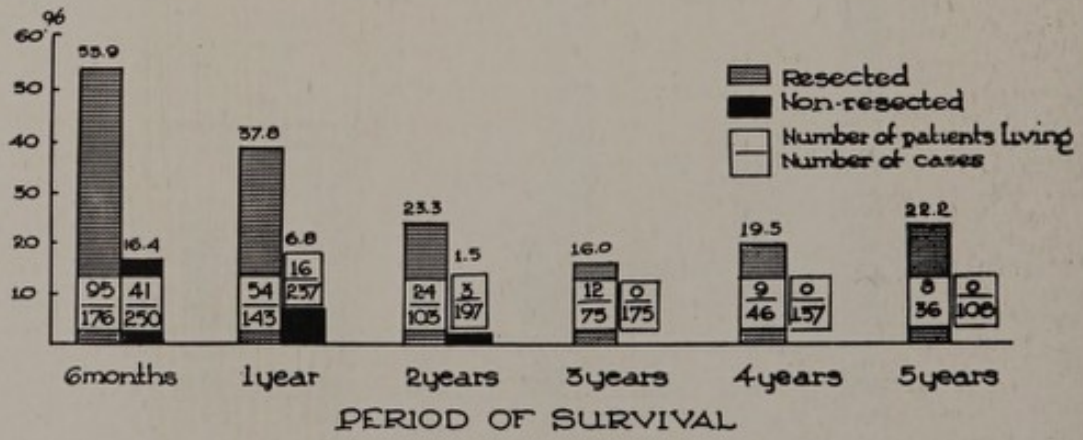

FIG. 13. Carcinoma of the lung; comparison of survival rates among resected and nonresected cases.

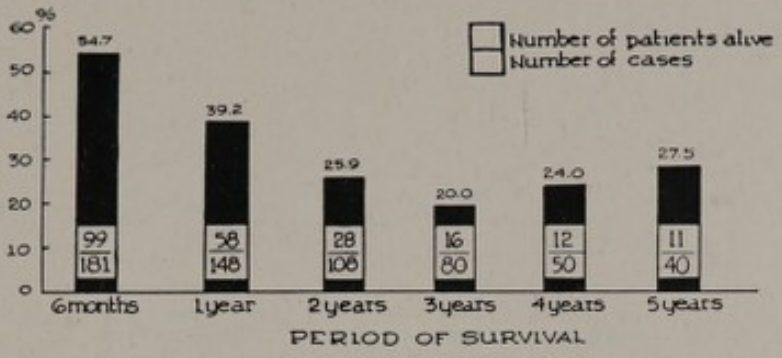

FIG. 14. Primary carcinoma of the lung; gross survival rate after resection.

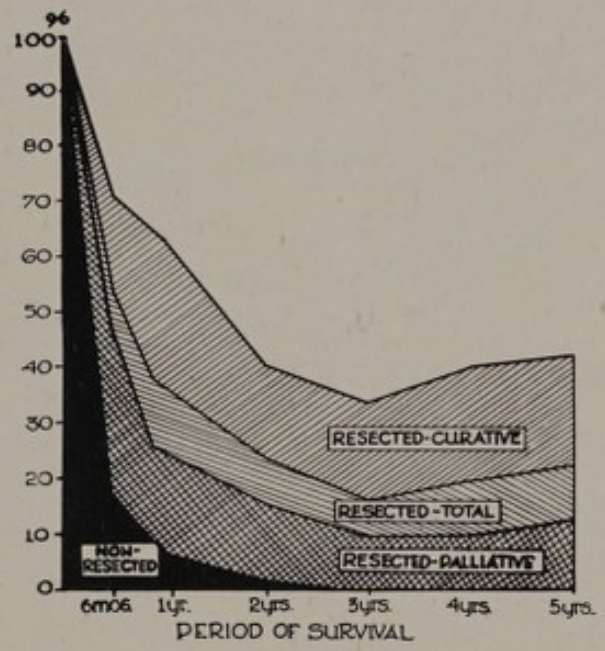

FIG. 15. Survival rates in carcinoma of the lung.

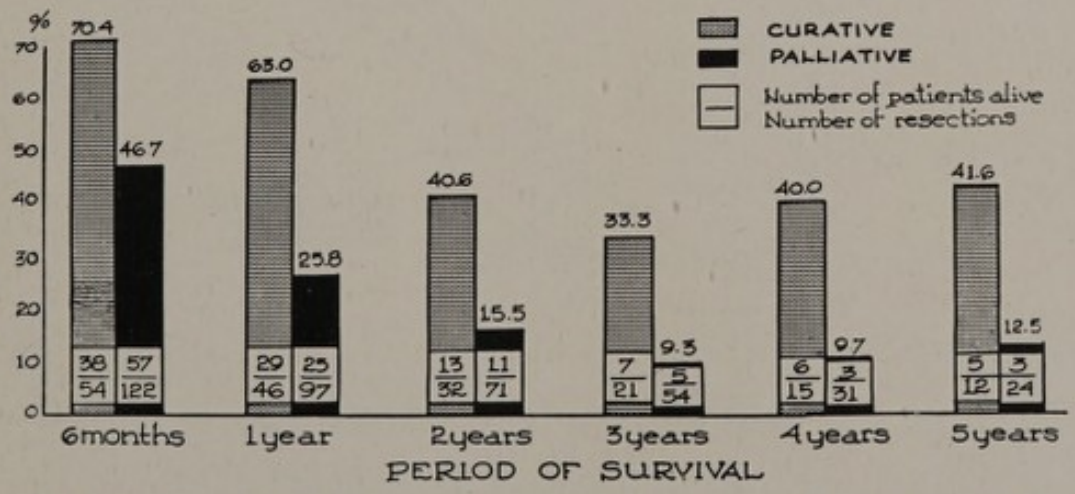

FIG. 16. Carcinoma of the lung; survival rates in 190 cases after palliative and curative resection.

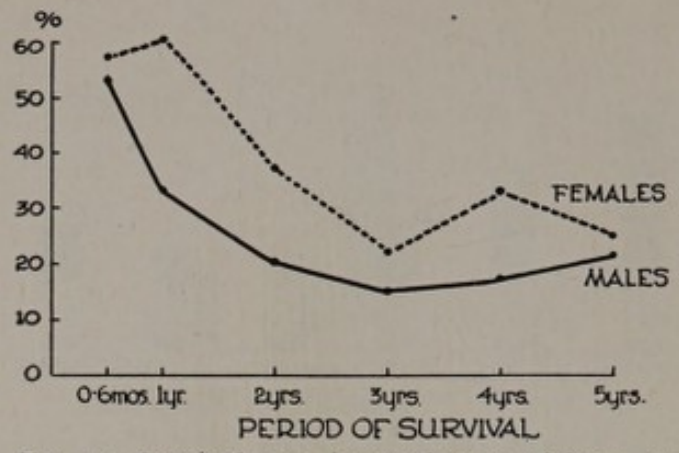

FIG. 17. Survival rate in carcinoma of lung according to sex.

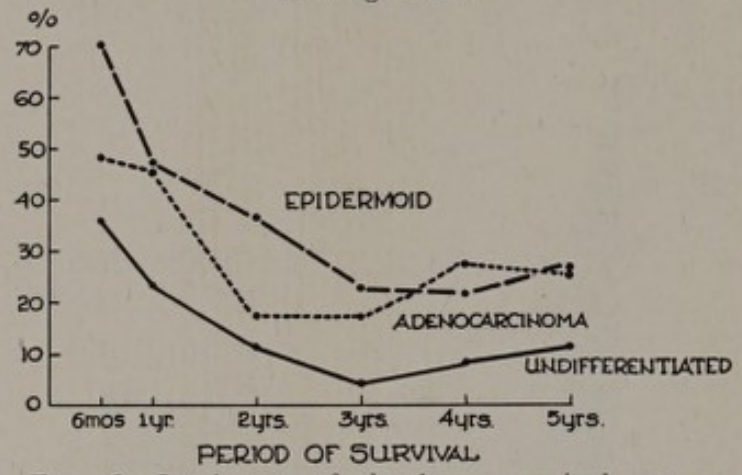

FIG. 18. Carcinoma of the lung; survival rate according to histological type.

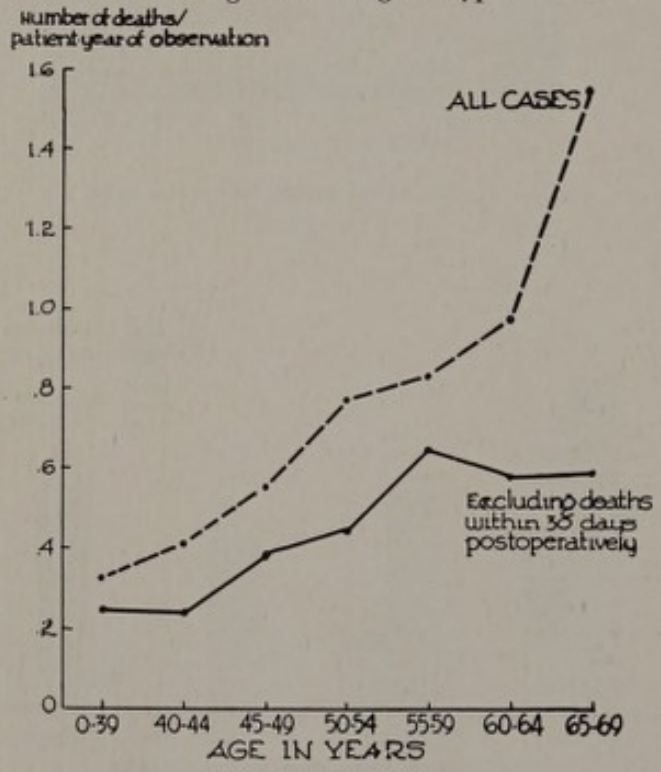

FIG. 19. Carcinoma of the lung; fatality according to age at time of operation.

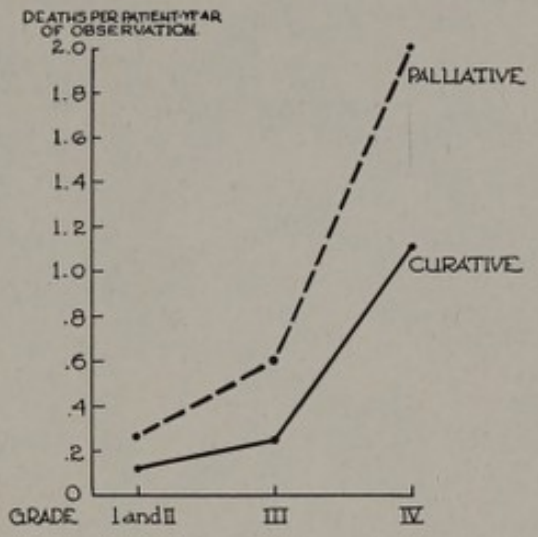

FIG. 20. Epidermoid carcinoma of the lung; fatality according to histological grade.

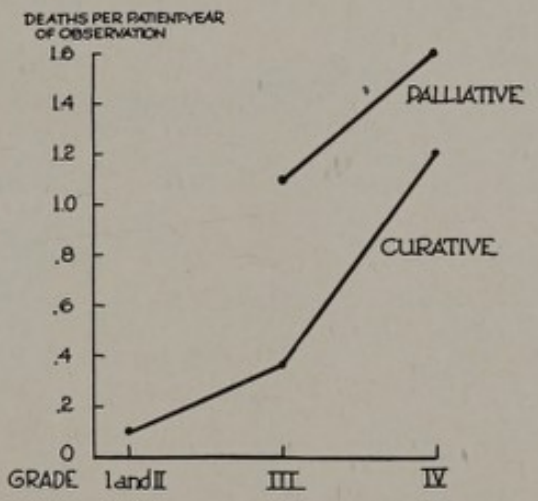

FIG. 21. Adenocarcinoma of the lung; fatality according to histological grade.

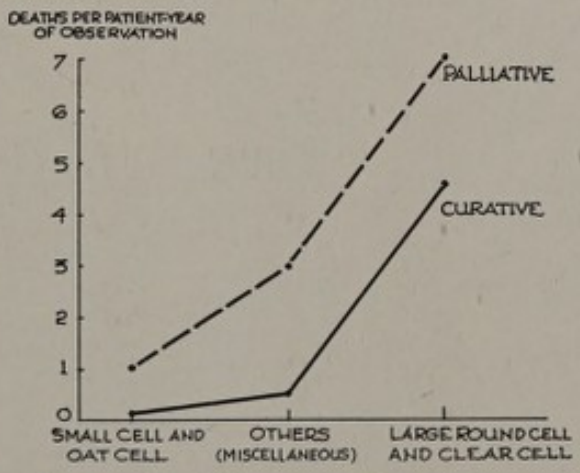

FIG. 22. Undifferentiated carcinoma of the lung; fatality according to histological type.

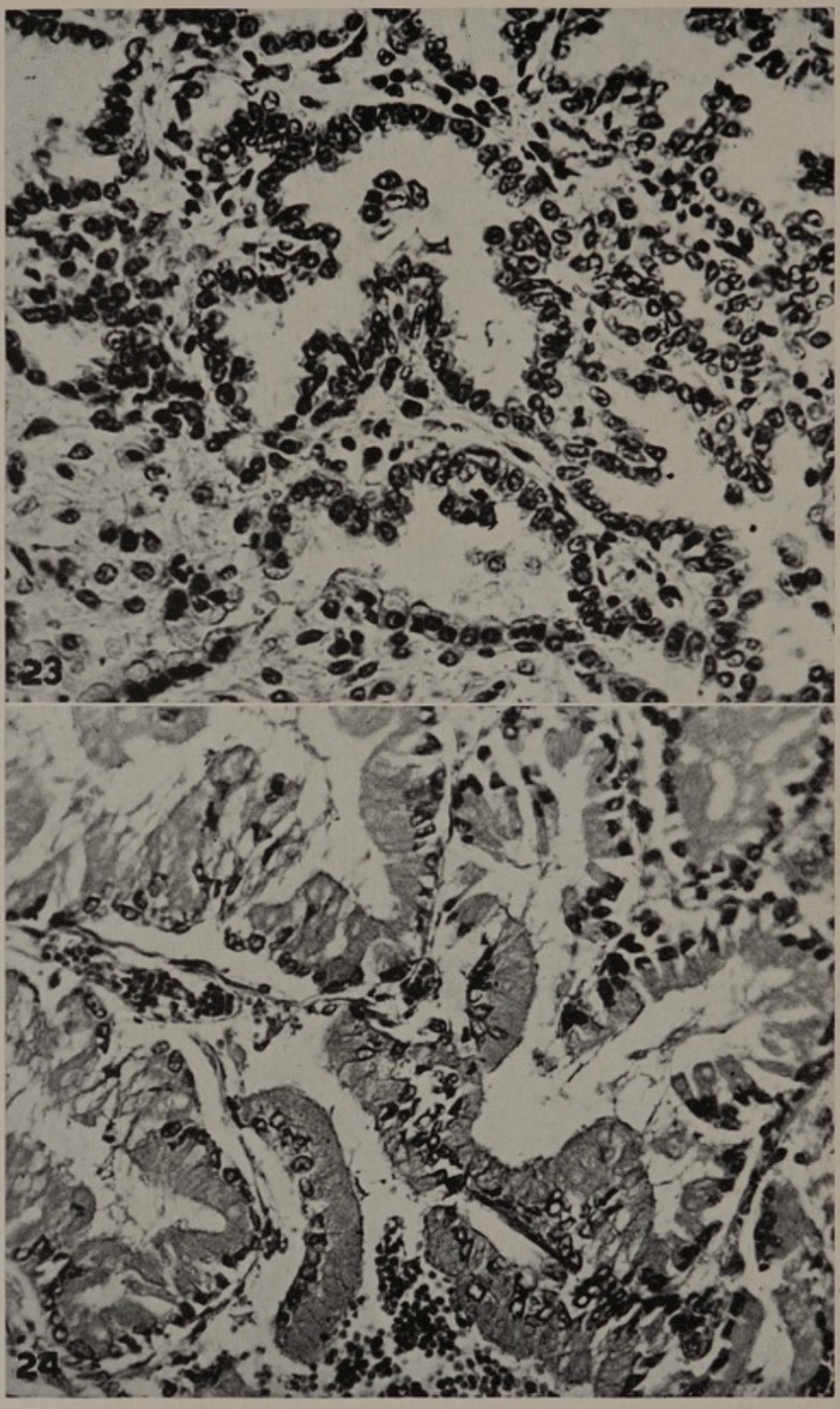

FIG. 23. Pulmonary adenomatosis in the sheep.
FIG. 24. Pulmonary adenomatosis in man. (Mucicarmine × 160.)

FIGS. 23, 24, and TABLE 1, p. 194 (*unpublished data:* Smetana and Swan): *Courtesy of the* Army Institute of Pathology.

for treatment. Four months of this delay was due to the patient, whereas six-months' delay was due to the physician.

The possibility that the increased incidence of carcinoma of the lung is due to increasing exposure to carcinogenic factors was suggested. It was pointed out that if significant extrinsic carcinogenic factors are present and could be controlled, the incidence of lung cancer would decrease.

A motion was made, seconded, and passed by unanimous vote that the American Cancer Society and the National Cancer Institute of the United States Public Health Service be requested to attempt co-ordination with the Heart Disease and Tuberculosis groups in developing co-ordinated mass chest roentgen-ray surveys for the early detection of these three types of disease.

Laboratory Studies

Inbred Mice and Lung-Tumor Incidences

A brief summary of the commonly used inbred strains of mice and the incidence of spontaneous pulmonary tumors was reported. Strain A has generally proved to have the highest spontaneous incidence, it being 80 to 90 per cent in animals living 18 months or longer. The incidence reported in the Swiss strain is 40 to 50 per cent, but this is an over-all incidence and if calculated for the 18-month age group would approach 80 per cent. Other strains were cited that are very resistant to the occurrence of lung tumors. Because the strains are highly inbred, the incidences remain comparatively uniform generation after generation.

Pathology and Histogenesis of Experimental Lung Tumors in Mice

The considerable difference between the pathological characteristics of these tumors and those of human bronchogenic carcinoma was emphasized. In mice, the spontaneous tumors are single or few in number, peripheral in location, and apparently arise from alveolar cells rather than the cells of the bronchi. The histogenesis is not apparent in the fully developed tumor. Two studies were reported, one of strain A mice with multiple tumors induced

by the application of a carcinogenic hydrocarbon at a remote point and another in which urethane was administered in drinking water or intraperitoneally in similar animals. In both studies, the animals were sacrificed relatively early, at short intervals, and serial sections of the lungs examined. Both studies indicated that these tumors arise from a preliminary alveolar hyperplasia, in the periphery remote from bronchi or bronchioles. The septal cells in one of these series showed a twenty-fold increase in the frequency of mitotic figures. At the same time, the epithelial cells lining the bronchioles showed no increase in the frequency of mitotic figures. None of the usual evidences of inflammation were present in the tissues of these animals. The question was raised whether early in the course of development of these lesions, the cells may represent proliferation of histiocytes. It is possible that the same cells may progress either in the direction of desquamated histiocytes or in the direction of neoplastic cells. It was suggested that cells may be found in human cancer tissues that apparently have the same potential capacity. Another study was reported that indicated that the cells of these tumors do not have histological reticulo-endothelial characteristics.

Extrinsic Factors Influencing the Development of Lung Tumors

The spontaneous occurrence of tumors of the breast or lung in mice is a characteristic of this species. Dr. John B. Murphy was acknowledged as the first investigator to demonstrate the induction of mouse tumors by carcinogenic agents. An early problem in the historical development of investigative work in this field was mentioned: whether the carcinogen acts (1) by direct contact with the susceptible pulmonary tissue or (2) by releasing a systemic tumor tendency in the susceptible animal. The evidence suggests that the action is by direct contact of the carcinogen with the pulmonary tissue.

All studies indicate that although carcinogens accelerate the neoplastic process and increase the number of pulmonary tumors observed, the incidence of such increased induction of tumors in different strains of mice follows closely the pattern of spontaneously developing tumors in the several strains. The susceptibility

of the pulmonary tissue in selected inbred strains of mice is such that they serve as excellent test animals for checking an alleged extrinsic carcinogen. A reasonably delicate assay of the potency of a carcinogen is possible because of the unique multiplicity of tumors formed. This fixed strain susceptibility of the pulmonary tissues to spontaneous or induced tumors is not paralleled by certain other tissues such as the hepatic. The multiple misleading problems that arise when external agents are introduced directly into the lungs, with the resulting inflammatory response and subsequent evidence of reparative bronchial epithelial hyperplasia, were noted. Subcutaneous and intravenous injections of the carcinogens have been used extensively, the intravenous method being somewhat more effective than the subcutaneous injection. It was pointed out that there must be an intrinsic susceptibility of a tissue cell for a carcinogenic agent to be effective. In a sense, then, carcinogens act as accelerators of tumor formation.

Polycyclic Hydrocarbons. Numerous hydrocarbons have been found to be active lung carcinogens. Significant quantitative effects have been detected if the doses are not less than four-times different one from the other. The common methods of inducing a carcinogenic response are (1) skin painting, (2) subcutaneous injection, and (3) intravenous injection; these methods are complementary. An agent may not show the same carcinogenic activity when it is tested by the several methods. Although not the only desirable method, intravenous injection is perhaps the best method to test exogenous pulmonary carcinogens. Here, the size of the particles of the carcinogenic agents is important, for the larger the particles, the more apt they are to be caught within the pulmonary capillaries and the greater is the observed carcinogenic effect. This factor must be controlled, if valid conclusions are to be reached. No difference has been noted in the excretion rate of a carcinogen by resistant animals as against the rate of excretion of susceptible mice.

It was concluded by one investigator that the pulmonary carcinogens act directly upon the pulmonary tissue; that a potential neoplastic process is present in all cells; that the carcinogens act by accelerating the inherent neoplastic tendency; and that such

acceleration is rapid and continuous following the single application of an active agent. With present methods of quantitative absorption spectrum analysis, it is usually impossible to detect the presence of the carcinogen in the tissues as early as one week after its administration.

An air-dust study was reported, the air dust having been obtained from several cities in the United States. With the methods used, no significant increase in the incidence of pulmonary mouse tumors could be demonstrated with the dust itself, although a significant increase occurred with an extract of the dust. Subcutaneous injection of tars extracted from these dusts induced sarcomas in mice at the site of injection in about 8 per cent of the animals.

Azo Compounds. Hemangioendothelioma was observed in the lungs of 41 per cent of strain C mice injected subcutaneously with *o*-aminoazotoluene. The order of response appeared to be associated with the site of injection. Classical lung tumors were also elicited with this compound. 3 : 4 : 5 : 6-Dibenzcarbozole has been demonstrated to be active in producing tumors in the lungs of strain A mice; dimethylaminoazobenzene (butter yellow) was inactive.

Bile Acids. Certain bile acids have been found to have a moderate carcinogenic activity.

Ethyl Carbamate (Urethane and other Esters of Carbamic Acid). The carcinogenic activity of urethane was originally discovered in animals to which urethane had been given as a sedative. It has its greatest effect in strain A mice. The order of response was the same whether the agent is given in single or in divided doses. The route of application was likewise immaterial. The variability of response of strain A mice to this type of carcinogen is so great that a large number of animals must be used to develop a significant pattern of behavior.

A significant increase in the incidence of tumors in the offspring of mothers to whom one dose of urethane was given just prior to parturition was reported. The number of tumors per mouse was very significantly higher, if the agent were given to the mother within twenty-four hours preceding parturition, than when given

one to four days earlier. Other studies demonstrate that 75 per cent of urethane disappears from the body within twenty-four hours.

Numerous related substances, e.g., a series of homologous esters and a series of *n*-alkylated derivatives of urethane, have been tested, and none has been found to be as active as ethyl carbamate.

Numerous other sedatives including ethyl alcohol, the barbiturates, chloral hydrate, and paraldehyde have been tested and found to be inactive.

Diet. Historically, low cystine diets were found to protect mice against the spontaneous occurrence of tumors. More recent studies with lung tumors indicate that this is a nonspecific result of the ingestion of a diet of inadequate caloric content. Where adequate caloric intake is maintained, such protection does not occur. It was concluded that a low caloric diet will give a partial but significant degree of protection to mice against the occurrence of spontaneous lung tumors.

Tobacco. Experimental studies were reported in which strain A mice were exposed to the inhalation of tobacco smoke four hours per day for twelve months. No increase in the occurrence of lung tumors was noticed. The tar from such smoke was painted on the skin and injected into the subcutaneous tissues and tar suspensions were injected intravenously and fed without the appearance of tumors in the lung or any other organ during the life span of the animal. It was pointed out that provided oxygen is present, polycyclic hydrocarbons of known carcinogenic activity are not formed on the combustion of tobacco. Oxygen is present in cigarette smoking.

A clinical review of 200 case histories of patients with bronchogenic carcinoma was reported suggesting that tobacco smoking is a significant factor in the production of human lung cancer. Lack of time prevented further discussion of this problem.

Industrial Factors. Whole-body continuous exposure to *gamma radiation* to a total dose of approximately 2400 r has been found to be a weak carcinogenic agent in strain A mice. *Ultraviolet irradiation* sufficient to produce skin tumors does not increase the incidence of pulmonary tumors in mice, suggesting that no car-

cinogen is formed in the skin and subcutaneous tissue. No cases suggesting the induction of bronchogenic carcinomas in human beings by roentgen-ray radiation have been reported.

Reference was made to the high incidence of carcinomas of the lung in men working in the Schneeberg and Joachimsthal mines. There is, however, insufficient evidence that such tumors are the result of irradiation with alpha rays emitted by radon. Arsenic and cobalt are also present in the dust of these mines and may be responsible in part for the increased incidence of cancer.

The incidence of reported cases of carcinoma of the lung in the *chromate* industry was cited. Eighty-nine cases have been reported in producing industries and nine cases in consuming industries. Preliminary laboratory tests with inbred strains of mice are under way.

The discussion on *silicosis* and *asbestosis* was postponed.

Intrinsic Factors Inducing Lung Tumors

That genetic factors are very important in the production of pulmonary tumors in mice is obvious from the marked difference in strain susceptibility. This has also been borne out in genetic analyses of the differences between the strains. The inheritance of the spontaneous tumor appears to involve genes identical with those necessary to the induced type. In both, multiple factor inheritance has been shown. Four identified genes have been shown to have an influence on the development of lung tumor, one of the most interesting of which is the lethal yellow gene, which also causes these animals to become obese.

Pulmonary Tumors in Species Other Than Man and Mice

A review was given of the world-wide occurrence of "pulmonary adenomatosis" or so-called "Jaagziekte." It occurs in many animals but is most common in sheep, in which it is a devastating and uniformly fatal disease. In certain countries, such as Iceland, it constitutes a serious economic problem. Large numbers of cases have been seen and studied in *sheep* in this country.

The disease is of multicentric origin and appears more commonly as a diffuse pneumonic type of involvement rather than as

the rare nodular form. There was considerable debate as to whether metastases have ever been demonstrated. Certainly such evidence is not abundant. There was disagreement among members of the Panel as to whether the disease is inflammatory in character or a definite neoplasm. Grossly, the lung is depressed rather than elevated over such lesions. No adequate studies have been made, to date, concerning the possible viral or infectious nature of the disease. It was suggested that it may be due to a specific susceptibility of pulmonary tissue and may be precipitated by a wide variety of nonspecific irritant agents. This disease in sheep offers certain promising experimental possibilities.

The disease occurs in humans, in whom it is also multicentric in origin. Cases were reported in which lobectomy has been followed by the recurrence later in the remaining portion of the same lung or in the other lung. The problem of correct histological classification is apparent in human cases. The incidence of this lesion among human pulmonary tumors was suggested as 1.3 per cent. Pathologists and other physicians are urged to be alert to the occurrence of this puzzling condition.

It was reported that urethane can produce a tumor in *rats* that is histologically similar to the spontaneous tumor occurring in mice.

Lung tumors similar to those of the mouse have been found in the *guinea pig*. They have occurred spontaneously and have followed chronic irradiation and the intravenous injection of the carcinogenic hydrocarbons, methylcholanthrene, and dibenzanthracene.

PANEL ON SOFT-PART TUMORS

Moderator, ARTHUR PURDY STOUT, M.D.
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Recorder, IRVING M. ARIEL, M.D.

Soft parts, in this discussion, comprised all the structures covering the surface of the body excluding the epidermis, the epithelial structures of the skin and all other epithelial tissues, the lymph nodes, and the bone. The nerves, but not the ganglionic or paraganglionic tissues, were also included. The soft supportive tissues of the retroperitoneum and mediastinum were included, but again, none of the epithelial or lymphoid tissues, ganglionic and paraganglionic structures, nor the mesothelium of the pleura, pericardium, and peritoneum.

The nomenclature utilized is one that traces the neoplasm, either benign or malignant, from the presumed type tissue of origin.

NOMENCLATURE

TYPE OF TISSUE	BENIGN	MALIGNANT
Fibrous tissue	Fibroma Fibromatoses	Fibrosarcoma
Adipose tissue	Lipoma	Liposarcoma
Smooth muscle	Leiomyoma	Leiomyosarcoma
Striated muscle	Rhabdomyoma (?) Granular-cell myoblastoma	Rhabdomyosarcoma Granular-cell myoblastoma
Blood and lymphatic vessels	Hemangioma (all types) Lymphangioma	Angiosarcoma, including hemangioendothelioma hemangiopericytoma Kaposi's disease
Synovial mesothelium	Synovialoma (?)	Synovial sarcoma
Lymphatic and reticuloendothelial tissues	Xanthoma Xanthogranuloma Giant-cell tumor	Lymphosarcoma (including mycosis fungoides) Reticulum-cell sarcoma Plasmocytoma
Mesodermal melanoblasts	Blue nevus	Melanosarcoma

Undifferentiated mesenchyme	Mesenchyme	Myxoma Mixed mesodermal tumor (mesenchymoma)
Heterotopic bone and cartilage	Myositis ossificans Osteochondroma	Osteogenic sarcoma Chondrosarcoma
Nerve sheath	Schwannoma (Neurilemoma) Neurofibroma	Schwannoma Neuroepithelioma (all types)

The question of whether *benign tumors of synovial origin* exist as pure neoplasms or whether they represent tumors probably of inflammatory or some other nature was discussed. Because it is possible to follow the sequence of the benign synovial tumor, starting as a villous hypertrophy (pigmented or nonpigmented) of the synovia, and then a fusion of the villi, which merge and form sessile-like masses that are indistinguishable from the usual giant-cell tumors of tendon sheaths, the conclusion was reached that these tumors were productive processes of hypertrophy rather than true neoplasia.

The diagnosis of *malignant tumors of synovial origin* is frequently difficult because of the large masses of spindle cells, which produce a picture akin to fibrosarcoma. However, if a careful search is made of numerous sections, clefts with cuboidal or flat cells surrounding these clefts will be seen and will give the clue to the nature of the tumor. Either the term "malignant synovioma" or "synovial sarcoma" is acceptable. Because of common usage, the term synovioma, if used, should be restricted to the malignant neoplasms of synovial origin and not used for the benign lesions unless the adjective "benign" is also used. This is important because in the past the pathological diagnosis of synovioma in instances of benign tumors has resulted in radical surgery including amputation. The term "xanthoma" or "giant-cell tumor of tendon sheaths and joints" should be utilized for benign tumors of this type even though these benign tumors may sometimes grow to large size, erode bone, and may be bilateral.

Tumors of Fibroblasts. The term "fibroma" is reserved for the small pedunculated tumors or the small fibrous nodules noted in the skin and occasionally elsewhere.

Desmoid tumors of the abdominal wall occur almost exclusively in women and are very frequently associated with pregnancy. There is a definite sex relationship; only one desmoid tumor of the abdominal wall has been seen in a male at the Memorial Hospital. After resection, if a recurrence develops, it is usually associated with a pregnancy or occurs immediately after the termination of pregnancy. The tumor is almost exclusively located in the lower abdominal wall inferior to the umbilicus. There were two instances cited in which such tumors of the abdominal wall were growing diffusely and castration resulted in cessation of growth.

Whether this tumor is a true neoplasm or represents a productive process related to increased estrogen titer is not known. It is the feeling that these are not true neoplasms.

Well-differentiated fascial fibrosarcoma is a neoplasm that resembles the desmoid tumors of the abdominal wall histologically, but is found in extra-abdominal locations, most frequently in the extremities as well as in the abdominal wall. These tumors occur in men almost as frequently as in women, although the predilection is still for the female sex. The tumors infiltrate and frequently recur after radical resection. They rarely metastasize. They may grow to huge proportions and may occasionally infiltrate the chest wall to produce a bulky mass that fills the chest cavity, or they may continue extending up an extremity until that entire extremity may be deeply involved by the neoplasm. They do not kill rapidly and cases are on record of patients who have borne this neoplasm from twelve to fifteen or more years.

There are two types of *hemangioendothelioma,* a benign and a malignant. The benign types are ordinarily seen in infants and it was suggested that they might be called capillary hemangiomas with endothelial-cell proliferation or benign hemangioendotheliomas. The term "hemangioendothelioma" has sometimes been used both for the benign and the malignant forms of the neoplasm, which has resulted in needless radical surgery for certain of the benign. Hence, in all cases of malignant tumors of this category, the term "angiosarcoma" may be used or the adjective "malignant" precede the term "hemangioendothelioma." The angiosarcomas should be subdivided further because the malig-

nant hemangioendothelioma is frequently more malignant then the malignant hemangiopericytoma.

Peripheral Nerve Tumors. The term "neurogenic sarcoma" should be deleted from the nosology of nerve tumors, and the cell type that comprises the tumor be used to designate it (i.e., schwannoma or neurilemoma). Most tumors designated "neurogenic sarcoma" in the past were either of fibroblastic origin or derived from the cells of Schwann. The latter should be designated "malignant schwannomas."

After a recent review of several hundred tumors previously classified at the Memorial Hospital as neurogenic sarcoma, most of the tumors were removed from this category and the remaining ones are now designated "schwannoma."

Lesions of neurofibromatosis may be designated as neurofibroma.

Research in Soft-Tissue Tumors

The administration of a carcinogenic azo dye, *o*-aminoazotoluene, into a certain strain of mice subcutaneously in the axilla resulted in hemangioendotheliomas in 30 to 40 per cent of the cases. Most of these tumors developed in the interscapular fat, but some arose in the fatty deposits throughout the body. Similar tumors could also be produced by administration of hydrocarbons or by exposure to ultraviolet radiation. Thus, different carcinogens could produce a single type of tumor.

If the azo dye is injected subcutaneously at the base of the tail, hemangioendotheliomas of the lung develop. If given in crystalline form in the axilla, tumors of the liver, lung, and fatty tissue are produced, but if given in olive oil, local tumors at the site of application are also produced. These local tumors are sarcomas and thus far have developed only after the administration of olive-oil solutions.

Thus, a given carcinogen under different conditions produced different types of neoplastic responses.

Harold L. Stewart demonstrated that the injection of a carcinogenic hydrocarbon may elicit neoplastic lesions from various tissues; that one tumor may be composed of one, two, or more

tissues (fibroblasts, endothelium, smooth and skeletal muscle, and nerves), and when transplanted, certain of the tissue characteristics such as the muscle cells may disappear and only fibrosarcomatous features persist.

If methylcholanthrene is injected into guinea pigs, liposarcomas develop within the fat in 20 per cent of the cases, but if the same chemical is injected into mice, liposarcomas of the fat are not observed, suggesting some species difference in the susceptibility of fatty tissues to undergo malignant changes.

Fibroblasts in tissue culture treated with 20-methylcholanthrene may become malignant and when transplanted to animals, a malignant sarcoma demonstrating all manifestations of malignancy will grow at the site of transplantation.

The sex incidence in hemangioendotheliomas is an interesting feature in animal experimentation. These angiomatous tumors were produced more frequently in females by the injection of azotoluene. If the males were castrated, the incidence increased significantly.

Stewart also referred to the tissue-culture work of Earle who has succeeded in isolating single cells in vitro and transplanting them.

The incidence of angioma of the human female is twice that of the male sex. Kaposi's hemorrhagic sarcoma differs from the other angiosarcomas in this respect, since it occurs almost exclusively in the male sex.

Margaret Murray discussed the *tissue culture of neoplastic cells.* A glomus tumor was cultured by Murray and the epithelioid cells identified as pericytes. This led Stout and Murray to classify the glomus tumor as an organoid vascular tumor form featuring pericytes and to suggest that other tumors that have a perivascular arrangement of cells but that are without the organoid arrangement of the glomus tumor should be classified as hemangiopericytomas.

Tissue culture is useful for diagnosis of soft-part tumors at four levels:

1. Without histological sections.
   Sympathicoblastoma

2. To enhance the characteristic of a tissue in aiding the histological sectioning technique.
   Hodgkin's disease
3. The histological sections are not clear.
   To differentiate between a liposarcoma, rhabdomyosarcoma, or fibrosarcoma
4. Tissue culture is not useful in epithelial tumors.
   Diagnosis depends upon topographical arrangement, which is lost in tissue culture.

Etiology

The development of malignant tumors from benign tumors, although extremely rare, may occur, and the surgeon should be conscious of that possibility. Examples were presented of fibrosarcomas developing from a fibroma or a cicatrix; malignant schwannoma developing in von Recklinghausen's disease; malignant fibrosarcoma developing as a result of radiation and possibly liposarcoma developing from lipoma. Two instances of malignant angiosarcoma that developed seven and eighteen years respectively following treatment for benign lymphangioma were cited. The question of sarcomas developing in burn scars was discussed, but none of the Panel had ever observed any. The fact that the carcinomas developing in burn scars are frequently of the spindle-cell variety has caused confusion in the literature and they have been erroneously classified as sarcoma.

No specific etiological factors could be presented. An occasional tumor of soft parts is seen in infants and suggests a congenital relationship.

No specific evidence that trauma is a causative agent in producing sarcomas was established and although this is suggested at times, there is always the possibility that the trauma may have injured a pre-existing tumor and so called attention to its presence.

Concerning age, sex, and race, nothing specific was brought out except that hemangiosarcomas are reputed to be more prevalent in women and two instances from Memorial Hospital were presented demonstrating their occurrence in the prepuberty state. The state-

ment was made that, as a rule, synovial sarcomas tend to occur earlier than fibrosarcomas.

Distribution of Soft-Tissue Tumors

The two most frequent sites of soft-part tumors are the thigh (including the gluteal and inguinal regions) and the retroperitoneum. Of 432 cases at the Memorial Hospital, the lower extremity was involved in 47 per cent, the trunk in 22 per cent, the upper extremity in 15 per cent; and all other sites in 16 per cent. The Mayo Clinic experience revealed that the thigh and the knee region were the most common sites of extremity sarcomas and that those of the thigh and buttock regions were usually the most malignant.

The histological variation, when compared to anatomical regions, reveals that the fibrosarcomas occur most frequently in the thigh and that the retroperitoneal region is a very uncommon site. The liposarcomas and the leiomyosarcomas are the most frequent tumors that occur in the retroperitoneal area. The liposarcomas in addition to being most prevalent in the retroperitoneum are also seen frequently in the shoulder and the pelvis. The possibility was suggested that these sites represent the location of embryological limb development and the fatty tissues in these sites are more prone to develop sarcomas than those in other locations.

Synovial sarcomas are limited almost exclusively to the extremities. According to Pack, they represent the most frequent malignant tumor seen in the hands and the feet. This was not the experience of other members of the Panel. Although they arise from synovial tissue, very few arise within the joint or tendon sheath itself but usually outside of them. Joints and tendon sheaths may be secondarily invaded.

The other types of soft-tissue tumors are observed too infrequently to permit an evaluation of their true anatomical distribution.

Growth and Spread

Soft-tissue malignant tumors usually grow slowly and a period of two to five years may elapse before they manifest themselves

clinically. The insidious local spread is a feature that permits their microscopic extension into contiguous structures beyond those that can be palpated clinically. This is probably an important factor that vitiates attempts to cure these lesions by local excision.

Metastasis usually occurs by blood-vessel dissemination and one of the most frequent sites of metastatic manifestations is the lungs.

Metastases to regional lymph nodes occurs with enough frequency in synovial sarcoma and rhabdomyosarcoma to warrant careful clinical evaluation, and appropriate therapy in the over-all treatment in these neoplasms. Lymph-node metastases are very rarely encountered in fibrosarcomas, liposarcomas, and malignant tumors of peripheral nerves.

Diagnosis

All tumors of soft parts except the very small ones should be biopsied before definitive therapy is instituted. The extremely small lesions may be locally excised leaving a wide margin and further therapy is dependent upon the histological variety of the neoplasm and the clinical course. The type of biopsy whether aspiration biopsy, incisional biopsy and frozen section, or incisional biopsy and paraffin section, depends upon the experience of available pathologists, the type of lesion in the patient, and the inclination of the surgeon. The disadvantages of aspiration biopsy are that many pathologists are not versed in this type of diagnosis and that it may only differentiate between a benign and malignant tumor without permitting the histological classification. The disadvantages of the formal biopsy are that these neoplasms may be very vascular and intractable hemorrhage may ensue; the capsule may be under pressure, and following biopsy, the rent may not be repaired, so that, if a long period elapses before definitive therapy is instituted, the fungation of the tumor may result. The advantages, of course, are that it permits a definitive diagnosis from an adequate section of tissue. The Memorial Group favored biopsy for those tumors in which the clinical appearance demonstrates that the treatment between two subvarieties would be the same and that the only question is one of establishing the malignant

nature of the neoplasm before therapy is instituted; in those instances in which it is desirable to establish the histology of the neoplasm in order to institute preoperative radiation (such as in certain instances of liposarcoma); or in inoperable cases in which radiotherapy is administered in the hope that inoperable lesions may be made to shrink and be rendered operable. There was no general agreement with this, others preferring incisional biopsy.

The problem of vein invasion and embolic tumor formation was discussed and a technique described whereby a tourniquet is placed proximal to the tumor site, a biopsy taken, frozen section made, and then if the extremity is to be amputated, another tourniquet is applied proximal to the first one, which has been left in place during this interval, and the amputation effected between the tourniquets. This is performed in the hope that emboli, which may have developed incident to the trauma of the incisional biopsy, may be prevented from transgressing the tourniquet barrier. Others expressed the opinion that this was an unnecessary and probably useless gesture.

Roentgenographic diagnosis of soft-tissue tumors may be helpful in instances of deep-seated lipomas because fatty tissue is less dense than water and a radiolucent area may be observed. It may also be used for diagnosis for deep-seated hemangiomas where phleboliths may be observed.

Certain patients, following surgical excision of sarcomas, may develop postoperative clinical symptoms of pulmonary disease, which is frequently diagnosed as localized pneumonia but which later proves to be metastases at the site previously considered to be pneumonitis. Autopsy findings have confirmed this observation; hence, the entity of acute pulmonary metastases has led certain members to ligate the main venous chanels as the first step in any major surgery of soft-tissue malignant tumors of the extremities. This type of acute pulmonary metastases does not exclude the possibility that tumor emboli may already be lodged in the lung when the patient presents himself for therapy, but it does focus upon the possibility that surgical trauma may induce showers of tumor emboli.

Treatment

The only method of treating this group of neoplasm is by surgery with certain assistance from radiotherapy.

Radiotherapy is considered to be valueless in rhabdosarcoma, leiomyosarcoma, and peripheral nerve tumors from the standpoint of effecting a complete cure. It is considered of no value in these conditions either postoperatively or in the treatment of pulmonary metastases.

The fact that tumors of the soft tissues appear to be radioresistant should not deter one from its use in certain instances. It is a known fact that most of the radiosensitive tumors are not radiocurable. Certain instances of synovial sarcoma and fibrosarcoma were presented that apparently had been cured by radiation therapy. Also certain cases of liposarcoma remained alive for ten to fourteen years with radiation therapy. Thus, radioresistance of a tumor should not deter one from using radiation in certain instances, and occasionally a radioresistant tumor may be radiocurable.

In instances of lymphosarcoma of the skin, a radiosensitive tumor, it has been demonstrated that 500 r administered in air results in 100 per cent recurrence in the irradiated area. Thus, the fact that a tumor is radiosensitive is no criterion for limiting the dosage.

The *surgical removal* of the neoplasm and the surrounding tumor bed is the paramount weapon by which these lesions may be cured. The extent of resection depends upon the histological type, the gross and microscopic extension of the tumor, and the previous efforts that had been attempted to eradicate the neoplasm.

For fibrosarcomas, a very wide local resection removing all the muscle involved from origin to insertion may be attempted. Very wide local resections for liposarcomas and myxosarcomas also may be indicated. In rhabdomyosarcomas, radical resection of all muscle groups involved may be attempted. When recurrence develops after such radical local resections have been attempted, resort should be had to amputation. Certain surgeons believe that

the best method of treating sarcoma of the extremities is by amputation at a substantial distance proximal to the tumor.

The point was discussed as to whether any sarcoma that has produced regional lymph-node metastases should be treated by amputation proximal to the metastases and radical excision of the lymph-node bearing area involved. An example of this would be a liposarcoma of the calf producing inguinal metastases treated by a hip-joint disarticulation and radical groin dissection.

Any patient who has had a local excision for a sarcoma and presents himself to an institution adequately staffed to handle cancer should have a wide surgical resection of the site from which the tumor had been excised locally. Tumors of the trunk including the retroperitoneal area should be treated by as wide surgical excision as is technically feasible and in certain instances of fibrosarcoma, synovial sarcoma, and liposarcoma postoperative irradiation may be instituted.

Prognosis

Although the over-all salvage rate of this group of tumors is not great, nevertheless examples of apparent cures of each variety of malignant neoplasms of soft tissues were presented.

The causes for the poor over-all results were attributable to: 1. Delay in diagnosis; culpability for delay usually lay with the patient in those instances in which a painless mass existed and with the doctor, when pain and no apparent mass existed. 2. Inadequate surgical intervention traumatically instituted without biopsy.

It was the feeling that marked improvement in prognosis could be attained by earlier diagnosis and the institution of more radical surgery.

PANEL ON BONE TUMORS

Moderator, DALLAS PHEMISTER, M.D.
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Recorder, MICHAEL BONFIGLIO, M.D.

ENZYMATIC HISTOCHEMISTRY OF BONE TUMORS

The discussion was opened by Gomori with a brief report of histochemical studies on fifty-three true bone tumors and, incidentally, on twenty-nine other sarcomas. Only portions of tumors sufficiently soft to be sectioned without decalcification were used. The glycogen content was found to be irregular. Some chondrosarcomas and osteoblastomas were very rich in it, others contained none. Giant cells of giant-cell tumors and other giant-cell lesions of bone were found to contain some glycogen.

Alkaline-phosphatase studies showed that this enzyme occurs in large amounts in young growing bone, preceding calcification in membranous bone, periosteum, and heterotopic ossification. Calcification of cartilage matrix takes place without alkaline-phosphatase action. In all osteoblastic sarcomas, large amounts of alkaline phosphatase were found, more than 200 units per gm. Sometimes the blood alkaline-phosphatase level is elevated. The highest activity was noted about blood vessels and in rapidly growing portions of the tumor, while necrotic or sclerotic central portions had low activity. Chondrosarcomas were found to be of two types: one resembling the osteogenic sarcoma, had a high alkaline-phosphatase activity, the other had none. Fibrosarcomas and giant-cell tumors showed no alkaline-phosphatase activity. Among the small round-cell sarcomas, two types of behavior were noted. The non-osteogenic sarcomas were invariably negative, the other types were positive; morphologically, the two were indistinguishable. One unusual case of myositis ossificans showed a very high alkaline-phosphatase activity. (Figs. 1 to 4.) Acid phosphatase was found to be absent from osteogenic sarcomas. It did occur in large amounts in the giant cells of giant-cell tumors, but as was brought

out by the discussion, this same property was shown by other giant cells of nontumorous nature such as osteoclasts, foreign-body giant cells and Langhans's cells of tuberculosis. Phosphamidase, an enzyme that was found to occur with marked regularity in epithelial cancers, was found irregularly in sarcomas.

The importance of alkaline-phosphatase activity in relation to tumors was stressed by Aub, since this activity can be quantitatively related to prostatic carcinoma and bone tumors. Apparently, a large amount of phosphatase must be present in a tumor before it appears in the blood stream. By a brief description of the growth of deer antlers, he stressed that much alkaline phosphatase can be produced in the antler without being mirrored in the blood serum and that the deer-antler growth was an excellent means by which to study phosphatase activity.

Beryllium-produced tumors in rabbits have been found to grow rapidly and to metastasize widely. Microscopically these were osteogenic tumors rich in alkaline phosphatase. The blood phosphatase was elevated only after the tumor became large. It was pointed out that in beryllium poisoning, beryllium is an inhibitor of alkaline phosphatase; but the phosphatase produced by beryllium-induced tumors was inhibited just as much as are other alkaline phosphatases.

The limitations of the histochemical methods were pointed out. The *p*H of the substances used should receive careful attention and that of tissue slices must be adjusted to the conditions at hand. This is often difficult because the proteins themselves are strong buffers and large margins of error occur when incubating tissue with substrate mixture. Inhibitors comprise another limitation, for they cannot be used with the idea that they completely inhibit an enzyme system. Usually they do not and residual enzyme activity persists. An example of such an error is the idea that cyanide inhibition of alkaline-phosphatase activity works by means of a heavy metal; no such mechanism is known in phosphatase activity. The oxidation mechanism is not well understood here. An attempt to repeat the work demonstrating two types of alkaline phosphatase in the blood by means of cyanide inhibition has been

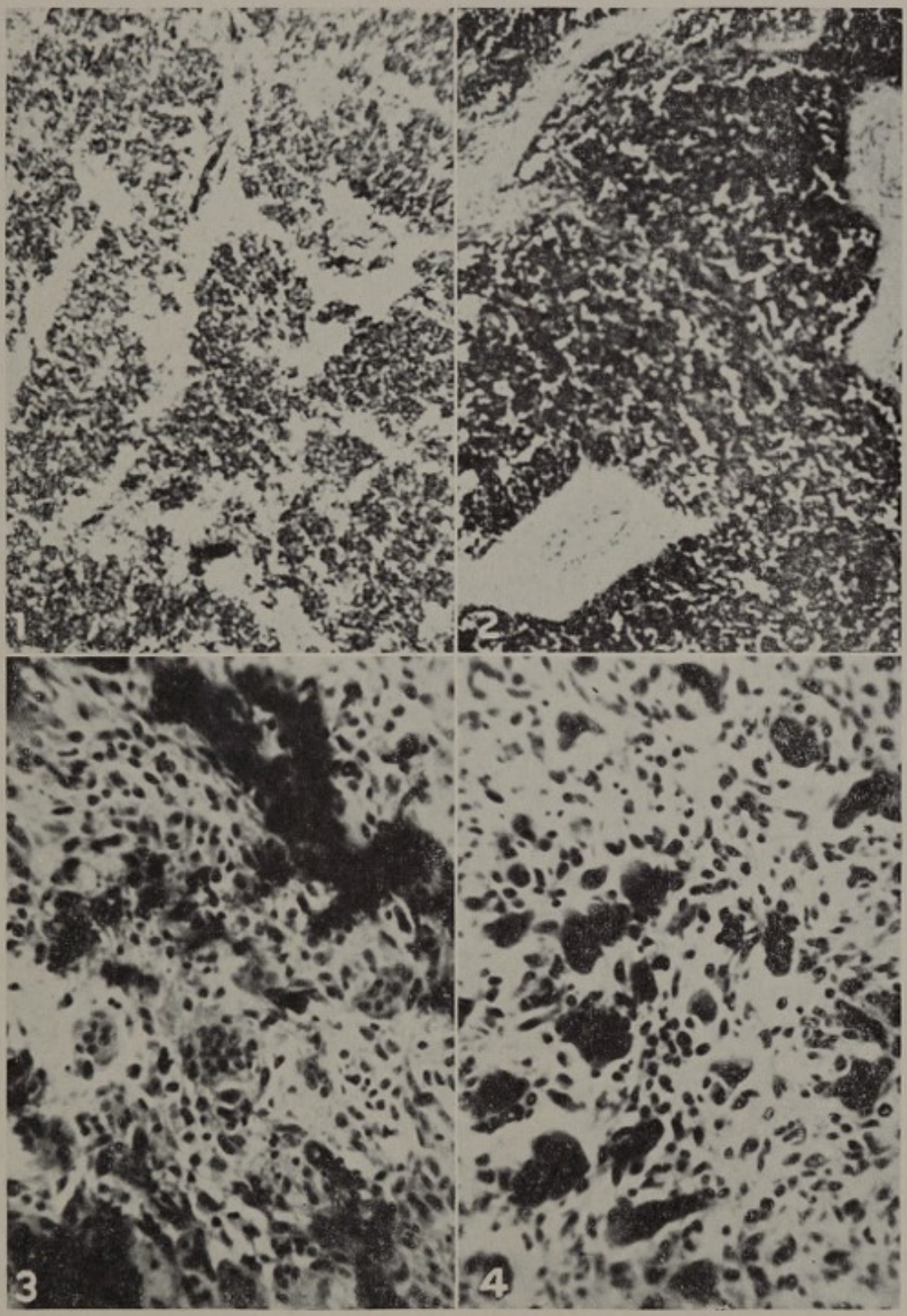

FIG. 1. Neuroblastoma metastatic to bone. Only capillary endothelium positive for alkaline phosphatase (black); tumor cells negative.

FIG. 2. Malignant synovioma. Tumor cells intensely positive for alkaline phosphatase.

FIG. 3. Giant-cell tumor of bone. Capillaries and strands of connective tissue positive for alkaline phosphatase.

FIG. 4. Another section of the same tumor. Giant cells positive for acid phosphatase.

unsuccessful, probably because incomplete inhibition of alkaline phosphatase occurred. The same remarks are applicable to other inhibitors and activators. Several cases were cited of both tissue and alkaline-phosphatase changes during a slowly growing phase of a tumor and later during an actively growing phase, proving they were related to the rate of growth.

Experimental Production of Bone Tumors

Roentgen-Ray Radiation

The earliest reports of osteogenic sarcomas in bones that have been irradiated were of those in animal experiments done about 1910 and in clinical accidents in which radium or low intensity doses of roentgen rays were given for a long time to patients for conditions other than tumor. An example of this is the use of radium injections in tuberculosis of joints. The occurrence of tumors in the radium-dial workers stimulated work on this subject. The question was posed as to why roentgen rays can produce tumors when they are useful in destroying them. The Memorial Hospital group discussed a recently reported series of eleven cases of osteogenic sarcoma appearing six to twenty-two years after therapeutic roentgen irradiation had been given. The original lesions treated were ossifying fibroma, benign giant-cell tumors, osteoid osteoma, bone cyst, fibrous dysplasia, and antrum infection. One of the tumors appeared in a rib in the field of irradiation given following a radical mastectomy for a breast carcinoma and another in the postnasal region following irradiation of a retinoblastoma. The total doses used were less than 2000 r to the bone in three cases, 25,000 r in another case, and the remaining from 3000 to 9000 r. The effect of roentgen rays on normal bone was discussed, and it was noted that with less than 2000 r, no immediate measurable effect was detectable. There were slight changes with doses of 2000 to 3000 r; with doses of 3000 to 4000 r, there was evidence of permanent damage. More than 5000 r to adult bone usually produced necrosis and disintegration. In chil-

dren's bone, longitudinal growth can be stopped by small doses, but bone survives doses up to 9000 r. The question of whether these sarcomas were spontaneous or induced was introduced, and it was decided that they were induced. The probability that the giant-cell tumors treated by roentgen rays were malignant from the start was considered remote because the interval between treatment and appearance of the osteogenic sarcoma was too long. Woodard presented a case to illustrate this last point.

It was pointed out that more of these cases are appearing now because only in the past twenty years has roentgen-ray treatment in large doses been widely used. Most of the members of the Panel have had cases of irradiation sarcoma appear in normal bones within the area of irradiation for other lesions. One case of extra-skeletal osteogenic sarcoma in the abdominal wall developed three years after irradiation of the regional lymph nodes in a case of a testicular tumor. The time interval was considered not surprising, since the earliest case of radium sarcoma occurred fourteen years after exposure. The statistics indicated that high doses were not necessary, in view of the fact that three of the cases cited had received less than 2000 r.

Radioactive Elements

Brues stated that it has not been possible to produce bone tumors in animals with as low a concentration of radium as has produced them in man because the life span of the animal is relatively short. It has been found that a large number of isotopes are bone seeking (e.g., calcium, strontium, barium, some of the rare earths, and some of the heavier rare earths such as plutonium). The animals that have been used are rats, mice, rabbits, and dogs. The formation of tumors in these animals is influenced by the site of application of the radioelement. The different species of animals reacted similarly to radioelement, which is not true of other carcinogens such as the hydrocarbons. The localization of the resultant tumors depends on the distribution of the agent, such as strontium in which 70 to 80 per cent of the tumors

are in the extremities, or plutonium in which 70 to 80 per cent of the tumors are in the axial skeleton. In rabbits, the jaw is the most frequent site of localization. There have been latent periods of at least three months before the tumor arises. Not much difference in effect has been noted between short and long periods of irradiation. The already noted cases in humans seem to agree with this.

The types of tumor produced in order of frequency are osteogenic sarcoma, fibrosarcoma, hemangioendothelioma, and rarely liposarcoma. Multiple bone tumors do appear in animals that have a high probability of tumor development. They also localize more frequently in the larger bones, such as the femur. Contrary to reports of others, chondrosarcomas or reticulum-cell sarcomas were noted in animals. By continued repetition of the dose of the radioelement, an increasing number of tumors could be produced. The latent period between the dose of radioelement and the appearance of tumor increases as the dose of strontium, for instance, is lowered, but it is not a linear function. At the highest dose, all of the animals get tumors, and with decreasing doses, fewer tumors appear. Both alpha- and beta-ray emitters were noted to act qualitatively the same in mice.

A discussion of the effect of radium on the human body brought out the fact that radium is present in very small amounts in all humans, but it was not known whether these amounts could influence the body to produce tumors following some type of injury which first lowered the threshold for carcinogen action (so-called threshold theory). Many of the Panel have had experience with patients treated with radium chloride for various diseases who developed osteogenic sarcomas many years later, usually ten or more. These patients showed evidence of radium poisoning as demonstrated by necrosis of bone and calcifications in areas near the epiphyseal ends of bone. The radium in these patients was localized early near the epiphyseal ends and later equally in cancellous and cortical bone. The work of Bloom was cited in which experimental osteogenic sarcomas showed the tumor to appear above the area of dead bone and in the regenerating area around the dead bone.

Classification of Bone Sarcomas

The following classification was submitted by the Moderator for discussion:

TISSUE	BENIGN	MALIGNANT
Bone	Exostosis, osteoma	Osteogenic or osteosarcoma
Cartilage	Chondroma hyaline chondroblastoma	Chondrosarcoma
Fibroblast	Fibroma	Fibrosarcoma
Giant-cell	Benign giant-cell tumor	Giant-cell sarcoma
Vascular	Hemangioma cavernous organoid	Hemangioendothelioma (Ewing's sarcoma?) Cavernous angiosarcoma
Marrow		Solitary myeloma and multiple myelomas
Reticulo-endothelial	(?)	Reticulum-cell sarcoma
Lymphatic	(?)	Lymphosarcoma
Fatty	Lipoma (?)	Liposarcoma (?)
Undifferentiated-cell (mesenchyme)	(?)	Mesenchymal-cell sarcoma (Ewing's sarcoma?)

Diagnosis and Treatment

It was pointed out that so long as the nature of *sarcoma* is not known, there will continue to be prolonged discussion about classification and reclassification of bone sarcoma. There is still confusion in the use of the term "osteogenic sarcoma." Ewing introduced it to include all sarcomas arising within bone. But it has come more and more to be used to designate only sarcomas arising from bone-producing cells and displaying some degree of ossification. In one respect, the much older term "osteosarcoma" is more appropriate, since it conforms to the nomenclature in use for sarcomas of other tissue types such as chondrosarcoma, fibrosarcoma, etc. According to Stout, osteogenic sarcomas are of two types, sclerosing and osteolytic. The amount of cartilage formation within the tumor determines whether or not he designated it as chondrosarcoma. Higinbotham uses the term "osteogenic sar-

coma" for bone-forming tumors, and differs from the old view of Ewing that all tumors that arose in bone should be called osteogenic sarcomas. He also believed that it was difficult to arrive at a final classification that would satisfy surgeons, pathologists, and roentgenologists. Many bone tumors contain all of the elements of bone so that the predominant tissue should determine the name. Jaffe designated those malignant tumors that form tumor bone or osteoid tissue in good proportion as osteogenic sarcomas. Like a fracture callus, all types of connective tissue may be present.

Chondrosarcomas have a different histological and cytological pattern. They are derived from cartilage and may undergo calcification and ossification but belong under a separate head. Those chondrosarcomas that demonstrate a high alkaline-phosphatase activity are probably osteogenic sarcomas that contain much cartilage.

The discussion of *chondromatous tumors* stressed the extreme importance of this group from the standpoint of malignancy. Single osteochondromas can become malignant, and an increase in size and the onset of pain are important symptoms to watch for. In the central chondromas, recurrences occurred if incomplete curettage was carried out. Also, in the single enchondromas of the phalanges, chondrosarcoma can develop but is not so common as in the multiple enchondromas. The central chondromas in the larger bones were considered the most hazardous benign tumor of bone. The Memorial Hospital group cited nineteen cases of benign chondroma that developed into malignant chondrosarcoma. An example of calcified chondromatous medullary defect was shown by Bennett who inquired about its possible significance. The central chondromas of the long bones and the pelvis required more radical treatment than did the peripheral phalangeal enchondromas. The difficulty of pathological and histological diagnosis of malignancy in the chondromatous tumors was stressed, especially that of making a diagnosis from one small field or segment of the tumor. Some of the histological criteria to be considered were that malignant cartilage cells have plumper nuclei, double nuclei, larger cells, and mitoses. Chondrosarcomas had rarely been noted to arise from the osteocartilaginous tumors

of dyschondroplasia or Ollier's disease. In the classification of bone sarcomas, Copeland tried to relate the type of tumor to the embryogenesis of bone. He called those chondrosarcomas that arose from osteochondromas and central chondromas a secondary type, and those that arose near the tendinous attachments a primary type. The primary type was a more aggressive tumor and metastasized early.

Most members of the Panel recognized primary *fibrosarcoma* of bone. Neither the primary fibrous type of tumor nor its metastases form either osteoid or bone, or display alkaline-phosphatase activity. Others classify the fibrosarcomas with the osteolytic type of osteogenic sarcoma.

The question of whether both benign and malignant forms of *giant-cell tumor* occur or whether one develops out of the other was discussed. There seemed to be general agreement that initially malignant giant-cell tumor did occur but if such were the case, they often were grouped with osteogenic sarcoma or fibrosarcoma. The term "benign giant cell tumor" was considered misleading by some, but others pointed out that it has saved many limbs from amputation. The giant-cell tumor usually occurs at the epiphyseal end of a long bone. It may thin the cortex and even expand it. It occurs in the age group of 20 years or over. Morton believes that when the tumor perforates the cortex and invades the surrounding tissues, it is malignant; if it metastasizes, it is not a giant-cell tumor. Johnson cited a case of a benign giant-cell tumor of the lower end of the radius that metastasized to the lung and had the same benign-appearing tumor in the metastasis. The treatment of these tumors has been surgical curettage and cauterization of the cavity wall with or without filling of defect with bone chips. A combination of surgery and roentgen-ray therapy is used by others, the roentgen rays being applied two or three months after operation and following the removal of the plaster dressing. Roentgen-ray therapy alone is used by some of the discussers. The danger of roentgen-ray therapy alone without biopsy was that the true nature of the tumor is not known and later another type of tumor may be noted. All members agreed that biopsy should be done but by the surgeon who is equipped to treat the patient. Needle biopsy

of bone tumors was not considered adequate. So-called giant-cell tumors of the jaw and facial bones as well as other giant-cell tumors should arouse suspicion of hyperparathyroidism and blood chemical studies should be undertaken in these cases.

A discussion of Ewing's tumor, or *hemangioendothelioma,* revealed that most of the Panel members were finding the term less and less applicable as time goes on. The hemangioendothelioma is considered a very rare blood-vessel tumor in bone and cavernous angiosarcoma was considerably more common. It was pointed out by Higinbotham of Memorial Hospital that hemangiomas of bone should be considered as dangerous as chondromatous tumors, since they have cases of hemangiosarcoma (or preferably angiosarcoma) arising from hemangioma. The term "Ewing's tumor" was considered poor because it described something other than an endothelial lining tumor. It has been retained because of common usage but needs continued clarification.

Lymphangioma or lymphangiosarcoma of bone was thought not to exist although the former tumor has been reported.

Reticulum-cell sarcoma was considered by most to arise from the reticulo-endothelial system, but there was disagreement as to whether this was a highly malignant tumor or not. They occur in ages 10 to 40 years. Higinbotham presented seventeen cases of such tumors in which a five-year survival occurred in ten. The treatment used by the Memorial Hospital was radiation and amputation in two cases, resection and radiation in one case, radiation alone in thirteen cases, and surgery plus toxins in one case. The tumor is very radiosensitive and doses of 2000 to 3000 r seem adequate to prevent local recurrence. These patients in general are in excellent physical condition, even though bulky tumors may be present. This is in contrast to patients with Ewing's sarcoma where the patients are usually quite ill. The view was expressed that some of the recoveries from Ewing's tumor probably belonged to the reticulum-cell sarcoma group. The answer is not yet final and a re-evaluation of Ewing's sarcoma was stressed. Stout stated that none of their cases of reticulum-cell sarcoma recovered. The confusion that exists between soft-tissue reticulum-cell sarcoma, lymphosarcoma, and reticulum-cell sarcoma of bone should

be clarified. The two former have been noted to metastasize to bone. The term "reticular sarcoma of bone" was introduced.

It was generally agreed that *liposarcomas* of bone did not occur. There may be lipoma-like accumulations, but they were thought by one panel member to be collections of fat in osteoporotic bone. The discussion of marrow tumors brought out minor difference of opinion as to whether solitary myelomas existed. Many instances were cited of solitary myelomas that existed for many years, even fifteen or more, before the development of multiple tumors. It was urged that serum-protein and other clinical studies should be carried out in patients with solitary myelomas. The term "myeloma of bone" was considered inadequate by some and "plasma cell myeloma" or "plasmocytoma" was preferred. Extraosseous plasma-cell myelomas of the nasopharynx have been noted by some to produce bone lesions years after the discovery of the tumor in the nasopharynx and the probable relationship between the two was considered.

Tumors consisting of undifferentiated mesenchyme probably occur more often in soft parts than in bone. The view was expressed that Ewing's tumor is an undifferentiated reticuloblast tumor in which pseudorosettes may be found. These pseudorosettes, however, must be differentiated from true rosettes which are found in metastatic neuroblastoma. Jaffe cautioned that undifferentiated and dedifferentiated epithelial tumors often simulated round-cell sarcoma of an undifferentiated type.

While *roentgenological diagnosis* is the earliest method we have of diagnosing bone tumors, it should not be considered accurate enough. Often the clinical history of pain precedes changes in the roentgenogram of a bone tumor. The physician should be alert to the possibility even if the roentgenogram is reported as normal and be persistent in the follow-up of patients, especially where symptoms continue. The radiologist may be of more help if an adequate history is furnished him. Care should be exercised in the study of the roentgenogram and repeated views taken where standard views are negative. Many of the roentgenological findings of bone tumors are seen in other bone diseases: destructive changes

in the cortex or medulla, periosteal changes, sclerosis, and eburnation of bone. Soft-tissue masses about such changes should lead to the suspicion of bone tumors.

PANEL ON TUMORS OF THE NERVOUS SYSTEM

Moderator, HARRY M. ZIMMERMAN, M.D.
College of Physicians and Surgeons, Columbia University, and Montefiore Hospital, New York, New York
Recorder, MARTIN G. NETZKY, M.D.

INCIDENCE OF DIFFERENT TYPES OF INTRACRANIAL NEOPLASMS

Eisenhardt gave the following figures from the Yale Brain Tumor Registry.

		No.	%	Total	%
Total verified tumors				2,209	100
Gliomas				944	42.7
Astrocytomas		274	29.0		
Fibrillary	171				
Protoplasmic	103				
Glioblastoma multiforme		242	25.6		
Medulloblastoma		90	9.5		
Astroblastoma		46	4.9		
Oligodendroglioma		38	4.0		
Spongioblastoma polare		35	3.7		
Ependymoma		29	3.1		
Ependymoma	18				
Ependymoblastoma	11				
Pinealoma		16	1.7		
Pinealoma	12				
Pinealoblastoma	4				
Ganglioneuroma		3			
Medulloepithelioma		2			
Neuroepithelioma		2			
Unclassified		167	17.7		
Atypical	30				
Cyst fluid	59				
Poorly preserved	5				
Degenerated	14				
Insufficient tissue	54				
Transitional	5				
Adenomas				395	17.9
Chromophobe		266			

	No.	%	Total	%
Acidophile	79			
Mixed Acidophile	33			
Basophile	2			
Adenocarcinoma	15			
Meningiomas			302	13.7
Neurinomas			191	8.6
All acoustic except one: of IX, X, XI, N.				
Congenital			117	5.3
Craniopharyngioma	95			
Cholesteatoma	13			
Dermoid	3			
Chordoma	2			
Teratoma	3			
Teratoma pinealis	1			
Metastatic			73	3.3
Carcinoma	55			
Hypernephroma	5			
Sarcoma	13			
Invasive			20	0.9
Carcinoma	9			
Myeloma	4			
Sarcoma	7			
Granuloma			45	2.0
Tuberculoma	33			
Gumma	12			
Blood-vessel tumors			45	2.0
Angioma arteriale	11			
Angioma venosum	6			
Hemangioblastoma	27			
Telangiectasis	1			
Sarcomas-primary			13	0.6
Papillomas			12	0.6
Miscellaneous			52	2.3
Unclassified	19			
Cysts	10			
Xanthoma	1			
Cranial	22			
Adamantinoma	1			
Angioma	3			
Osteoma	15			
Osteochondroma	3			

In the discussion of these figures, it was brought out that intracranial metastases have a higher incidence in the general population. Cushing's figures are low because he refused to operate upon these tumors.

Zimmerman pointed out that glioblastoma multiforme is apparently increasing in incidence. In most series, at present, it represents around 50 per cent of all gliomas. Autopsy series may have an unduly high percentage of this highly malignant neoplasm.

All gliomas occur more commonly in men than in women in

the ratio of 60 : 40. This sexual difference is more prominent in glioblastoma multiforme where the ratio of men to women may be 2 : 1. Davidoff stated that the reverse is true in meningiomas. Spinal meningiomas occur more frequently in women in the ratio of 2 : 1. Zimmerman commented that experimental tumors grow better in males. This is best seen in the growth of ependymomas, with which takes occur in only 20 per cent of females but in almost 100 per cent of males. Further studies of the mechanism of this sexual difference are necessary.

Davidoff gave figures on eighty cases of metastatic carcinoma upon which he had operated. There were only a few more men than women in this series. The most common primary sites were in descending order of frequency: lung, breast, kidney. Routine chest roentgenograms were advocated in the study of all adults with intracranial symptoms. In his series of spinal metastases, the order of frequency of the primary site was: breast, lung, rectum.

The question of grading of tumors by the new Mayo classification was briefly discussed by the Panel. The consensus of opinion was that the classification has not been studied enough. The subdivision of oligodendroglioma into four grades seems too extensive and arbitrary. On the other hand, the range of tumors from astrocytoma to glioblastoma multiforme may well require more than four grades. The ultimate value of any grading system will depend upon careful comparison with follow-up findings.

On the question "does operation make a tumor more malignant?" the Panel felt that intracranial tumors take on increased malignancy in only rare instances. Dedifferentiation can occur, as when a meningioma becomes sarcomatous, but this too is rare. An error can be made when the neurosurgeon biopsies the edge of a glioblastoma multiforme and finds only the surrounding astrocytic reaction. If then at autopsy, a glioblastoma multiforme is found, this is not evidence of an alteration of the tumor, but rather of failure of the biopsy.

Scharenberg felt that the usual hematoxylin-eosin methods are inadequate and that the use of the silver carbonate technique is desirable.

Early Diagnosis of Intracranial Tumors

Electroencephalography, pneumoencephalography, and arteriography, were discussed. Davidoff opened the discussion by stating that the pneumoencephalogram is valuable to show the presence of an intracranial mass. In metastatic tumor particularly, however, it fails to indicate whether the lesions are solitary or multiple. Exact localization is not always given by this method. On the other hand, he felt that in many cases, the electroencephalogram gives evidence of localization and of multiplicity of lesions. The arteriogram is similarly valuable in showing multiple lesions and localization. He felt that air studies are necessary in mid-line lesions but are less valuable in hemispheral tumors.

Most of the other members of the Panel considered the electroencephalogram the least valuable of the three methods. All were agreed that each method was of value in particular instances. Scarff stated that both the nature of the lesion and the location determine the electrical pattern. A slow-growing neoplasm producing little edema yields little electrical changes. In the presence of edema, electrical changes are more prominent. It was agreed by all that the hazard to the patient was greatest with the air study, least with the electroencephalogram, and intermediate with the angiogram.

All agreed upon the importance of protecting the angiographer. Davidoff stated that his method for protection of the neurosurgeon was to keep a lead curtain around the neck of the patient, and to use different men in making successive angiograms.

The problem of handling a patient who is about 40 years of age, who has a seizure for the first time, and who is without neurological signs, was discussed. It was agreed that the diagnostic tests should include an electroencephalogram and an air study. If these are negative, the patient should be followed both clinically and by repeated electroencephalograms. Clinical judgment must then be exercised as to further management. Sweet cited twenty cases in which the air studies were negative and an electroencephalographic focus found (data of Robert Schwab and John Abbott).

The Panel next considered whether early diagnosis was necessary in the handling of intracranial neoplasms. Adams pointed out that gliomas are not cured in most cases, and that neoplasms such as meningiomas are so slow-growing that immediate operation is not necessary. Sweet, however, asked whether a delay in operating on gliomas might not account for failure to cure. It was agreed that while the necessity for immediate intervention in gliomas was not comparable to that of metastasizing tumors elsewhere in the body, diagnosis should be made as soon as possible. After the tumor has been localized, the question of immediate operation must be determined by the effect that it would have on the patient. This would be dependent upon the type of tumor and the location, as well as upon the presence or absence of symptoms. Thus, a meningioma should be removed immediately; a tumor in the speech area should not be removed; and in the absence of symptoms, most members felt it would be better to wait.

In the discussion on the incidence of seizures as an initial symptom no agreement was reached on which types of intracranial neoplasms showed this more frequently.

Diagnosis and Localization of Intracranial Tumors

Radioactive Isotopes

Moore presented the work of the Minnesota Group in the use of radioactive isotopes for the *diagnosis and localization of intracranial tumors through the intact skull.* They used radioactive diiodofluorescein injected intravenously in 1-mc. doses. One to two hours later, the head is explored with a Geiger-Müller counter at symmetrical points on either side of the head. Since tumors take up greater amounts of the radioactive material, it is possible to triangulate the area of increased radioactivity. General, higher counts were found on the entire side of the lesion. In twenty-six cases, eight tumors were correctly localized; two tumors were closely but not exactly located; in six, no focal lesion was found, and one was demonstrated by other methods, including operation: seven tumors were missed or incorrectly localized; one tumor was localized only as to the proper hemisphere. It is felt that if a tumor

is larger than 20 cc. and if radioactivity in the tumor is 2 : 1 compared to nontumor areas, the neoplasm can be localized. Difficulties occur in the following conditions:

1. Perineurial fibroblastomas take up very little of the radioactive material.
2. Occipital neoplasms are more difficult to localize because there is more overlying muscle tissue and perhaps because the tumors are smaller there.
3. There is no dye uptake in abscesses.
4. Necrotic zones take up no radioactivity.
5. Subdural hematomas have little uptake.

It was found that metastatic tumors have a high uptake; glioblastoma multiforme may concentrate the material as high as twenty-nine times more than normal tissue. In cerebrovascular accidents, there is high uptake but no definite localization.

Selverstone reported the work of the Harvard Medical Group on the use of radioactive phosphorus in *localizing brain tumors at operation*. The P^{32} employed has a half life of 14.3 days and a maximum range in tissue of about 7.5 mm. Doses of from 0.8 to 3.6 mc. are given intravenously, preferably at least twelve hours before operation. A probe Geiger-Müller counter of the same size as a ventricular cannula was successfully employed in twenty cases by virtue of the fact that the P^{32} appears in high concentration in human cerebral tumors when compared to normal brain. The method had been particularly valuable in determining the precise localization of deep-lying brain tumors. There appeared to be an optimum time interval between twenty-four and seventy-two hours after injection in which they obtained the most satisfactory activity ratios. Concentration in glioblastoma at comparable time intervals appeared to be distinctly higher than in astrocytoma in most instances. The activity ratio appeared to be considerably higher in the more cellular meningiomas than in those that were predominantly fibrous. Metastatic tumors and chromophobe pituitary adenomas also showed high uptake of P^{32}. In three cases of subsequently proved tumor, none was localized by this method. In one of these, the patient proved to have diffuse glioblastosis and no

differentiation between various areas was possible. In another, the tumor was small and was missed by the exploring needle. In the third, the needle was not inserted deeply enough to reach a tumor invading the mid-brain.

Use of Fluorescein at the Time of Operation

Normal brain tissue takes up little fluorescein. Brain tumors, by and large, have a high uptake. Hence, the brain tumor is an ideal situation for visualization of nonradioactive sodium fluorescein under ultraviolet light. Moore uses the material intravenously in concentrations as high as 20 per cent (one gram). It is given slowly, usually in 5 per cent concentration in 20 cc. of fluid.

Moore also presented an evaluation of the clinical use of radioactive diiodofluorescein in the preoperative diagnosis of brain tumors.

Tumors correctly localized	17
No tumor localized, none demonstrated by angiogram, ventriculogram, or craniotomy	10
Correct localization (but vascular or demyelinization rather than tumor)	4
Close to, but not exact localization	2
Located as to hemisphere only	1
Tumor present but not located	13
Tumor localized but not found by operation, ventriculogram, or angiogram	2
Incorrectly localized	1
Abscess not localized	1
Cholesteatoma not localized	1
Total	52

Details of radioactive dye test on Mrs. J. L., April 15, 1948. 1-mc. dye at 2:00 P.M.

TIME	SITE	COUNTS/MIN.
4:05	Background	19
4:20	Left frontal (just supraorbital)	48
4:24	Right frontal (just supraorbital)	42
4:18	Left posterior frontal lateral // to floor	35
4:15	Right posterior frontal lateral // to floor	40
4:28	Left earline 45° toward floor	40
4:33	Right earline 45° toward floor	39
4:43	Left parietal 45° toward floor	48
4:40	Right parietal 45° toward floor	51
4:47	Left occipital // to floor	38
4:53	Right occipital // to floor	45
5:10	Left at apex of pinna // to floor	50
5:15	Right at apex of pinna // to floor	36

Conclusion: Negative for tumor.
Operation: Ventriculogram normal; left angiogram normal; multiple sclerosis.

Details of radioactive dye test on Mrs. M. F., February 16, 1948. 1-mc of I^{131} at 1:30 P.M.

TIME	SITE	COUNTS/MIN.
	Background	20
4:00	Left forehead	58
3:50	Right forehead	77
4:10	Left frontal top	60
4:05	Right frontal top	67
4:17	Left earline top	86
4:24	Right earline top	115
4:34	Occipital mid-line	45
4:40	Left occipital lateral	41
4:45	Right occipital lateral	42
4:55	Left lateral earline	42
5:00	Right lateral earline	45
5:05	Right lateral, 45° toward floor at earline	43
5:13	Right lateral, 45° toward floor post frontal (3 to 5 cm. anterior to previous reading)	73

Conclusion: Highest activity on right side, near mid-line, and anterior to earline about 3 to 5 cm.

Operation: Parasagittal posterior frontal meningioma. Before operation, radioactive and fluorescein dye intravenously; four hours later tumor fluoresced only slightly.

Biopsy: Normal brain tissue—31 counts per minute; tumor—93 counts per minute.

Details of radioactive dye test on J. H., 11 years old, December 5, 1947. Radioactive diiodofluorescein, 0.6-mc., given intravenously at 11:00 A.M.

TIME	SITE	TOTAL	COUNTS/MIN.
2:55	Lateral right frontal	397	59
3:00	Right earline	302	36
3:10	Left earline	202	46
3:15	Left forehead	366	52
3:20	Right forehead	481	75
3:25	Right frontal	401	59
3:40	Left frontal	321	31
3:30	Mid-line (earline)	254	30
3:45	Mid-line frontal	404	60

Operation: Tumor, right frontal lobe.

Pathology: Weight equaled 73 gm.; microscopically, ependymoblastoma.

The only side effect noted has been nausea and vomiting, which can be avoided by slow administration. Photographs of the visualization of tumors under ultraviolet light were demonstrated. If liver function is normal, the dye is rapidly excreted.

Davidoff felt that coloring of the cerebrospinal fluid and dura interfered with the method. Zimmerman showed that in experimental animals, the dye could be seen in saliva, dental enamel, the gallbladder, the biliary system, and thence into the gastrointestinal tract, and the urine. In experimental gliomas, he found the greatest uptake of the dye thirty minutes after injection, with little present after sixty minutes.

The attempt of Grant, some twenty-five years ago, to measure

changes in electrical resistance with needles in brain tumors was noted.

Treatment

Use of Radioactive Isotopes

Erickson discussed the use of radioactive phosphorus in the treatment of gliomas. The work is in its initial stages, hence the present statements represent mostly preliminary observations. Radioactive phosphorus is used at present for inoperable gliomas, such as pontine spongioblastoma polare, or multiple glioblastomas. It has been given orally or intravenously, no advantage having been obtained from arterial injections. At first, the dosage was 10 mc. in one to two doses, but later it has been given in doses of about 1 mc. each day for ten days.

Thirty patients including eighteen with glioblastomas have been treated. The radioactive phosphorus appears to have some therapeutic effect but is definitely not curative. If too much is given, a depression of bone marrow with agranulocytosis will result. It is not known whether radioactive phosphorus itself causes swelling. Fractionation of phosphorus in normal brain tissue shows the radioactivity to be greatest at first in the acid-soluble fraction, with later increased uptake in the nucleoprotein fraction.

Selverstone stated that in normal brain, the specific phosphorus radioactivity in the lipid and acid-soluble fractions was of the same order of magnitude. In tumor material, they are each at least five times higher than in brain. The ratio of specific activities of the nucleoprotein fraction of tumor to brain is greater than unity but less than in the other fractions. It should be emphasized that these ratios were obtained in material from which specimens had been taken only twenty-four to forty-eight hours after injection of P^{32}. He also showed figures for the uptake of radioactive phosphorus in the different organs at autopsy. In three cases the uptake by glioblastoma was high, but was higher in liver and bone marrow. The potential danger to the latter organs was discussed. Sweet cited a case in which sufficient radioactive phosphorus was given to cause granulocytopenia, but the glioblastoma continued to grow. There

was no answer at present to the question of the use of roentgen-ray radiation together with radioactive isotopes.

Radium and Roentgen-Ray Therapy

The use of radium in the treatment of all types of tumors was agreed to be unsatisfactory.

The Panel also agreed that in certain rare instances, gliomas had been cured by roentgen-ray therapy, but at the cost of extensive damage to normal brain tissue. There was no agreement as to the appearance of heavily irradiated normal brain tissue: it was variously described as that of a formalin-fixed brain or of a putty-like white matter with coagulation necrosis and normal gray matter. Amyloid degeneration was reported in two cases by Scharenberg. Zimmerman stated that heavy irradiation in normal brain tissue caused ganglion-cell destruction with little gliosis. He found fibrosis within the tumor.

All agreed that medulloblastoma was the intracranial neoplasm most sensitive to roentgen-ray therapy, although the treatment is not curative. The Panel was unable to agree on the value of irradiation in glioblastoma multiforme. Davidoff stated that a rare case of this tumor improves, but that it is impossible to predict which case will benefit. Grant and Semmes pointed out that the benefit of roentgen-ray therapy given postoperatively can easily be confused with the benefit conferred by decompression.

There was no agreement concerning the value of roentgen-ray therapy in blood-vessel tumors. Scarff cited two cases of pinealoma that were cured by roentgen rays.

The roentgen-ray treatment of pituitary adenomas was discussed extensively. Most members felt that the eosinophilic type responded better than the chromophobe variety. All agreed that failure of vision was an indication for operation. Without loss of vision and with no optic atrophy, irradiation should be used. Constant check of visual fields during therapy is necessary. Irradiation should also be given following operation. Watts cited two cases of adenoma with cure.

None of the members of the Panel subscribed to the use of roentgen-ray therapy at the time of operation.

The Panel agreed on the necessity for standardization of the numerous factors entering into the use of roentgen-ray therapy. This would include a study of the number of portals, machine voltage, dosage, and the various physical factors.

Cerebral Lobectomy

Grant, Scarff, and Odom agreed that an entire lobe should be taken out if it contains tumor. The only exceptions would be if the tumor involved the motor cortex or the dominant speech center. Davidoff commented that subtotal removal of tumor, leaving obvious neoplasm behind, was still compatible with long survival in certain cases. The Panel agreed that a higher mortality results from conservative therapy and that even in glioblastoma an internal decompression should be done when lobectomy is not feasible. All members also agreed that a patient who presented a picture of glioblastoma multiforme clinically should nevertheless be operated upon, because one in ten such cases may have a resectable tumor.

The Panel considered the question of implanting tumor in normal brain tissue by a needle that had passed through tumor. Moore stated that such implants were commonly observed in mice. Zimmerman stated that he had never seen tumor cells within a needle tract. He commented that brain tumors almost invariably do not metastasize but spread locally. Tumor extension is usually farther than can be detected by naked-eye observation.

A glioma can be implanted on dura. In one case, a glioblastoma has been found in the lung of man and in cervical nodes in two cases.

Functional Localization in the Brain with Special Reference to Areas That May Be Removed with or without Totally Disabling Sequelae. Erickson cited a case of removal of the anterior 6 cm. of the dominant temporal lobe without production of aphasia. Sweet cited one case of removal of most of the left temporal lobe. There was an eleven months' survival with little intellectual change as measured by the Wechsler-Bellevue tests. A second similar case showed a relatively normal performance except in spelling, twelve

days after operation. Scarff found no changes in personality or intellect in eight patients in whom the frontal lobe on the dominant side had been removed, nor in eight others after removal of the nondominant side. He commented on the fact that no aphasia occurs in frontal lobotomies that apparently pass through the motor speech center. He concluded that there is no element of dominance in the frontal lobe.

Two cases were presented by Sweet in more detail, and illustrated, to show that temporal lobectomy of the dominant temporal lobe was not necessarily followed by gross incapacitation of the speech functions.

In the first case, patient R. M. entered the hospital and was operated upon in coma. The expanded left temporal lobe was removed in toto; it contained a large glioblastoma multiforme, the extirpation of which was, to gross appearances, complete. Thirteen days postoperatively, the battery of performance tests, including the Wechsler-Bellevue tests, showed a degree of language impairment that would not be consistent with the capacity to earn a living (continuous line, Fig. 1). Within less than three months, however, the performance had improved to the level shown by the discontinuous line in Fig. 1, and this was consistent with the patient's return to his original position as a foreman and tallyman in a lumber yard, in which job he had to make numerous simple mathematical calculations correctly.

In the second case, patient F. M. had seizures pointing to a lesion in the temporal region. Figure 2 shows the operative field of this patient, who proved to have an astrocytoma of the left temporal lobe. Lobectomy was carried out along the dotted line indicated in the photograph, just beneath the Sylvian fissure and just anterior to the anastomotic vein of Labbé.

Figure 3 reveals that the patient, preoperatively (October 27, 1948) had a performance that was, for the most part, in the upper half of the range of normal. Repeat testing, twelve days after the left temporal lobectomy, showed very little deterioration and a level of ability consistent with good earning power as well as normal social adjustment.

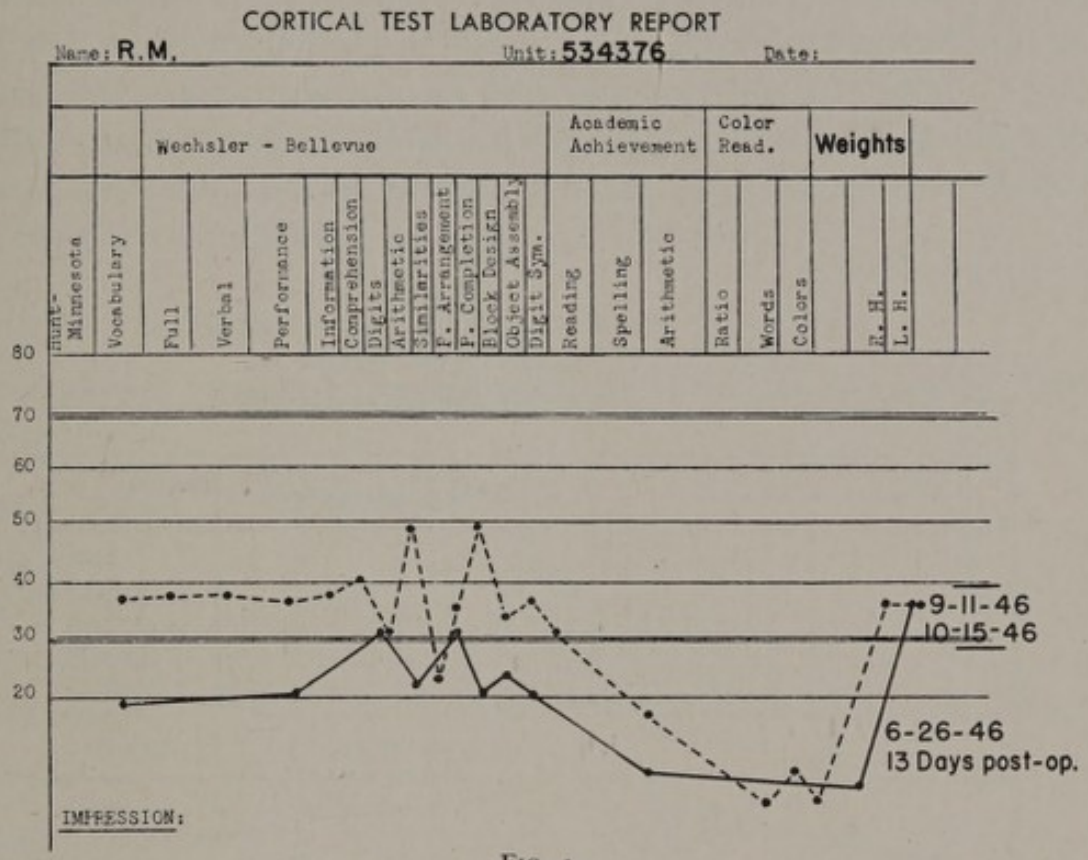
CORTICAL TEST LABORATORY REPORT
Name: R.M.
Unit: 534376
Date:
Wechsler - Bellevue
Academic Achievement
Color Read.
Weights
Minnesota
Vocabulary
Full
Verbal
Performance
Information
Comprehension
Digits
Arithmetic
Similarities
P. Arrangement
P. Completion
Block Design
Object Assembly
Digit Sym.
Reading
Spelling
Arithmetic
Ratio
Words
Colors
R. H.
L. H.
80
70
60
50
40
30
20
9-11-46
10-15-46
6-26-46
13 Days post-op.
IMPRESSION:

FIG. 1.

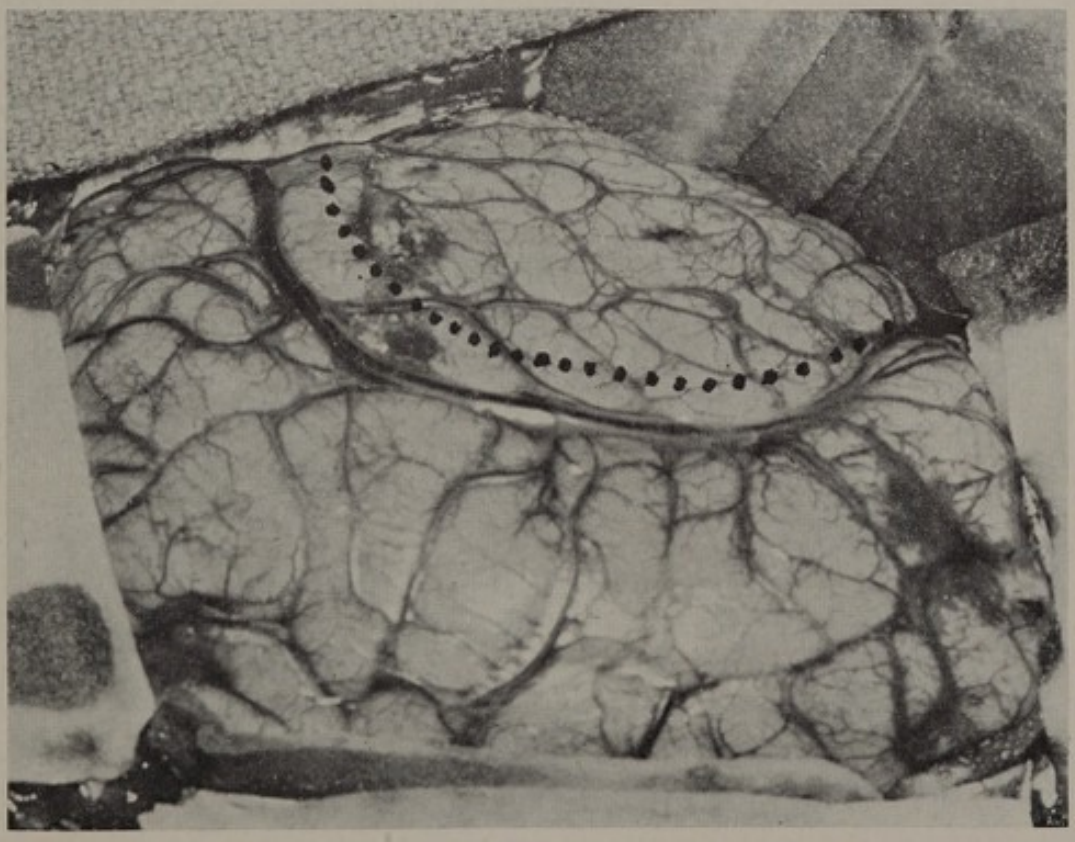

FIG. 2.

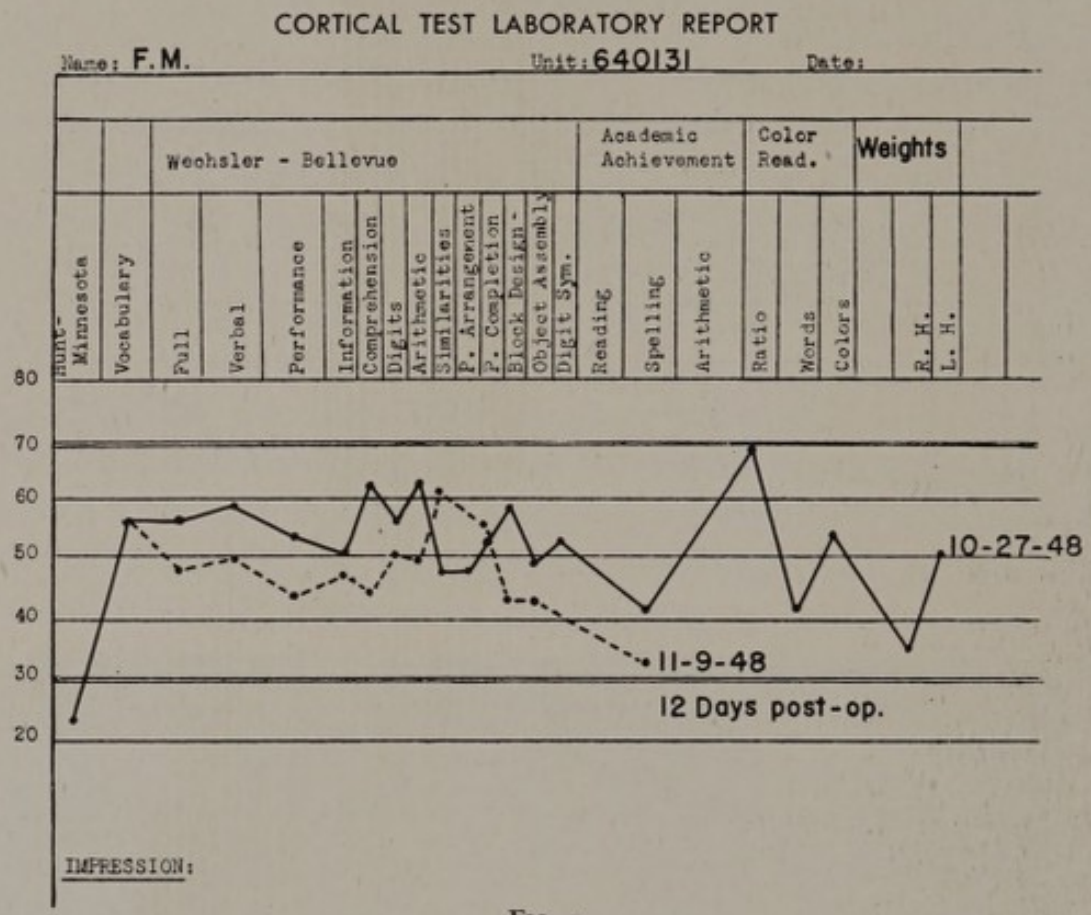
CORTICAL TEST LABORATORY REPORT
Name: F.M.
Unit: 640131
Date:
Wechsler - Bellevue
Academic Achievement
Color Read.
Weights
Hunt-Minnesota
Vocabulary
Full
Verbal
Performance
Information
Comprehension
Digits
Arithmetic
Similarities
P. Arrangement
P. Completion
Block Design
Object Assembly
Digit Sym.
Reading
Spelling
Arithmetic
Ratio
Words
Colors
R. H.
L. H.
80
70
60
50
40
30
20
10-27-48
11-9-48
12 Days post-op.
IMPRESSION:

FIG. 3.

Both patients had strongly dominant left cerebral hemispheres, and the excellent compensation that ensued following removal of the temporal lobe indicates in each instance that lobectomy of the dominant temporal lobe is not necessarily a hopelessly mutilating procedure. Patients with tumors therein should be given a chance for cure or amelioration by extensive resection.

Survival with or without Surgery

Eisenhardt reported the following survival periods in the Yale series:

Longest, Ferguson, 42 years; Zekvind, 37 years
Gliomas
Astrocytoma
Cerebellar
Ferguson, 42 years
Mulry, 30 years (complete)
Pickett, 33 years plus
Cerebral
Gorman, 27 years (protoplasmic)
Pons
Crowder, 8½ years (massive roentgen-ray therapy)
Spongioblastoma polare
Steinmetz, cerebellar, 21 years
Kirchner, 20 years
Oligodendroglioma
MacDonald, 23 years (frontal with mural nodule)
McKillop, 17 years (temporal with mural nodule)
Ependymoma
Orchard, 24 years (extremely radical operation)
Doucette, 24 years (well until sudden death with septicemia)
Medulloblastoma
Folmskea, 14 years (roentgen-ray therapy for two years)
Astroblastoma
Petherick, 21 years
1923, 1st operation
1943 and 1944, operations. Recurrence. Death, 1944
Meningioma
Shallow, 35 years
Turrell, 29 years
Corbitt, 31 years in 1941
Acoustic
Adams, 31 years to 1940
Wing, 25 years (age 81 years)
Saneoro, 28 years
Ramsey, 23 years (age 74 years)
Bohne, 26 years
Craniopharyngioma
Kimball, 19 years
Rosen, 25 years
Armstrong, 20 years
Keeney, 15 years

Special
- Trapp, 34 years
  - 1914, cyst evacuated
  - 1948, cyst with mural nodules

Chromophobe
- Kohn, 32 years (sphenoidal approach; later radium)
- Drangle, 30 years (death from coronary occlusion)
- Song, 30 years (sphenoidal approach)
- Beasley, 26 years
- Sazarowitz, 26 years

Acidophile
- Zehino, 37 years (sphenoidal approach)
- Mossmaud, 23 years
- Troop, 21 years (sphenoidal plus frontal approaches)

The members agreed that only infrequently was operation on glioblastoma multiforme followed by survival greater than one year. Operation appeared to be indicated, however, in all cases, since relief of headache was often achieved. No data are available on the survival time of patients with and without operation and with various types of operation. Adams emphasized the need for obtaining such information. A happy note was struck by the long survival of some of the Cushing cases in the series reported by Eisenhardt. Zimmerman reported a case of glioblastoma multiforme in the Montefiore series with survival for fourteen years from the time of the first symptom; another with an eight-year survival; two with seven-year survival; and one with a six-year. Scharenberg cited two cases with ten- and six-year–survival periods.

Davidoff discussed survival in cases with metastatic intracranial tumors. With decompression only, the majority of cases survived less than six months; none survived more than a year. With excision of the tumor, the survival rate is, by and large, equally poor. However, rare cases can survive as long as six years. He felt that operation on metastatic intracranial neoplasms was worth while if the tumor were solitary. Spinal metastases almost invariably have a short survival period.

Reporting of Cancer of the Nervous System in the Literature

This topic was not discussed. It is suggested that survival time should uniformly be reported from time of first symptom.

Chemistry of Tumors of the Nervous System

Folch-Pi commented on the difficulties in this pioneering work, in which it is still necessary to develop new methods and to establish control levels for lesions other than brain tumors as well as for brain tumors.

In its earliest stages of development, the brain tumor is similar in composition to normal brain. Brain tumors were found to contain fewer lipids than are present in normal white and gray matter. There are no cholesterol esters in normal brain tissue, but large amounts are present in tumors. Only small amounts of carbohydrate-containing lipids are found in tumors and sulfur-containing lipids are not present. In general, this pattern is more developed in glioblastoma than in astrocytoma. The brain tumor is similar in composition to normal brain in its early stages of development.

Folch-Pi discussed the problem of fractions of lipids that are at present unknown in nature. The proteins of brain tumors were briefly discussed. Pi did not believe that the presence of myelinated fibers in slow-growing tumors would account for the chemical differences reported. Zimmerman suggested the use of transplanted experimental tumors outside the brain as a source of material uncontaminated by brain constituents. The value of chemical studies in helping to determine which radioactive substances would localize in various tumors was commented upon.

Treatment of Intractable Pain from Extracranial Neoplasms

Chordotomy. Scarff reviewed a series of bilateral chordotomies, from the Neurological Institute, for relief of pain resulting from abdominal and pelvic carcinoma. One third of these cases gave a completely satisfactory result, in that pain was relieved and there were no side effects. In one third, the pain was relieved but there were either effects on bladder function or motor weakness. In one third, the pain was not relieved. Grant felt that he had obtained better results, since only 10 per cent of his cases had motor involvement and 20 per cent permanent damage of bladder control. He feels that a two-stage procedure for bilateral chordotomy results in

less bladder involvement. His operation is done at C5-6. In his experience, poor results are obtained with chordotomies in the thoracic region. The indication for the procedure is inoperable carcinoma with intractable pain below the level of the chordotomy. Burning pain may develop postoperatively as a complication. Davidoff felt that even with relief of pain, the patient may be unhappy, and drug addiction will continue. He raised the question of combining this procedure with lobotomy.

Lobotomy. Scarff reported thirty-three cases of unilateral prefrontal lobotomy performed for the relief of intractable somatic pain. Of the patients, 66 per cent operated upon had been completely relieved of their pain; 18 per cent had been partially relieved; 15 per cent had been unrelieved. The follow-up period ranged from one month to ten months. Careful psychometric and psychiatric appraisals were carried out before and after operation in a large percentage of the patients; these tests indicate that the operation has no adverse effect on intellect or personality. An unforeseen side effect of the operation has been its facilitation of the termination of severe narcotic addiction due to pain, without withdrawal symptoms or signs. Typical cases were cited in the report, and a statistical summary of results was given. The operation is accompanied by little or no shock and can be performed on the most debilitated patients in whom other types of operative intervention could not be tolerated. There have been no operative fatalities. The patients usually require only five- to seven-days' hospitalization after operation.

Watts presented a number of cases of carcinoma with pain in which bilateral lobotomy was done. He feels that extensive cutting on both sides is too much for a patient who has a short life expectancy. In six cases in which orbital quadrants only were cut, the results were good in five, and fair in one. Using the transorbital procedure, results were good in one case, fair in one, and poor in one. He feels that the procedure is not so useful in the treatment of pain as in the treatment of psychiatric conditions. Cases with unilateral lobotomy must be followed longer. Unilateral lobotomy is not accompanied by mental change, and if results are good over a longer period, this procedure may be of value. Davidoff reported

twenty cases of bilateral lobotomy with sixteen good results, but extensive mental change.

Value of Experimentally Induced Brain Tumors

The experimentally induced brain tumors were considered from two standpoints: their value as a means to study histogenesis and as an aid in therapeutic approaches.

Zimmerman presented a motion picture showing the early neurological signs of brain tumors induced by the intracerebral implantation of methylcholanthrene in mice. These animals develop a motor weakness and ataxia starting after 100 days following implantation. Gliomas develop in these animals, and the tumors can then be transplanted for further study. The type of glioma that develops depends on the location of the pellet. Medulloblastomas develop in the cerebellum. Ependymomas develop when the pellet is near the ventricular surface. Glioblastomas, astrocytomas, etc., develop in the cerebrum. Histologically, these tumors are indistinguishable from those of comparable human material. If the pellet extrudes extracranially, the animals develop rhabdomyosarcomas with production of an enlarged head. These tumors develop much more rapidly than the gliomas. When transplanted, the induced tumors may become more malignant with successive transplants, then become stable after about twelve transplants. An induced tumor may contain two or more glioma types. These mixed tumors can be transplanted and ultimately grown as pure types, somewhat in the manner of bacterial subcultures. No tumors of ganglion cells have ever been encountered.

The early induction of the neoplasm has been shown to result when methylcholanthrene or one of its derivatives enters the cell. Such a cell then becomes neoplastic.

Martin commented on the difficulties encountered in growing human glioma material in the anterior chamber of the eye of experimental animals. Zimmerman confirmed the low percentage of takes of such material. He found that slower-growing neoplasms usually grow less well in the guinea-pig's eye.

PANEL ON EPIDEMIOLOGY, INCLUDING ENVIRONMENTAL AND OCCUPATIONAL CANCER

Moderators, W. C. HUEPER, M.D.
National Cancer Institute, Bethesda, Maryland
LOWELL J. REED, SC.D., PH.D.
Johns Hopkins University, Baltimore, Maryland
Recorders, FRANK R. DUTRA, M.D., AND
B. AUBREY SCHNEIDER, SC.D.

The Moderator opened the Panel on Environmental and Occupational Cancer by stating its purpose to be "to evaluate the relationships of environment and occupation to the incidence of cancer." The last few years have brought an impression that occupational cancer is considerably more common than has been heretofore believed. It is now known that there are certain substances of definite carcinogenic activity to which workers are exposed, and there are other substances in the environment that are suspected of being carcinogenic. Some of these have thus far only produced tumors in experimental animals, but under certain conditions they might also induce neoplasms in man.

CANCER OF THE URINARY BLADDER

With the development of the aniline dye industry, the problem of occupational cancer of the urinary bladder had its birth. At first, it was believed that a number of dye intermediates could cause bladder cancer, but gradually the number of suspected carcinogens has been diminished. Although aniline has been the substance most often blamed for producing bladder tumors, it now seems highly probable that it is incapable of doing so. Certainly no tumors have occurred in aniline workers in the United States, two groups of whom have been studied extensively.

Beta-naphthylamine is the most potent, and may be the only, substance in the dye industry that produces bladder tumors. It has been proved to cause bladder tumors in men, and experimental

studies have revealed that dogs exposed to the substance develop carcinoma of the bladder within a relatively short time. The exposure to this substance in industry is almost entirely by inhalation and cutaneous contact.

Alpha-naphthylamine has been suspected of producing some bladder tumors, but it has not produced tumors in experimental animals. The technical grades of this material, as used in the dye industry, are all contaminated with as much as 5 per cent of the highly active beta-naphthylamine. Some tumors have been reported in workers exposed to technical grades of alpha-naphthylamine containing beta-naphthylamine as an impurity.

The evidence that benzidine is a cause of bladder tumors is not so good as that in the case of beta-naphthylamine. Fewer human cases have been reported, but some of these, notably from one German plant, appear to have involved exposure to benzidine alone of all the suspected agents. Attempts to produce bladder cancer in dogs with benzidine have failed. However, liver tumors and jaw tumors have been produced in rats with the material.

It appears that man requires an exposure of from two to twenty years to beta-naphthylamine to develop carcinoma of the bladder. The cancers can be prevented by properly limiting exposure, by cleanliness of the employees, and by an active medical control program. The latter should include examination of the urine for blood at least once every three months. Annual cystoscopy of exposed employees has also been suggested. At least 1000 cases of bladder cancer have been traced to exposure to aromatic amine exposure. The actual number is considerably higher. England's industry alone has more than five hundred cases.

Cutaneous Cancer

The first portion of the discussion on cutaneous cancer was concerned with problems in the petroleum industry. In 1942, samples of certain fractions of oils that had been cracked by a catalytic process were submitted to the Barnard Hospital. It was suspected that these agents, or some of them, might be capable of inducing cutaneous carcinoma. One of these fractions, when its physical properties were investigated, was found to contain one or more

substances that resemble anthracene. This fraction was found to have a high carcinogenic potency when tested on mice, while another sample which contained none of the anthracene-like material was capable of inducing some tumors but had a relatively lower carcinogenic potency.

It has been learned that fractions from the catalytic process which have a boiling point lower than 700°F. are not capable of producing cancer. Thus, the gasoline, kerosene, home heating oils, and diesel fuels obtained from this process appear to be innocuous. There are several fractions that distill above 700° that are capable of inducing cancer in the skins of mice. At the Sloan-Kettering Institute, some 200 selected samples have been tested. Of these, some of the high-boiling fractions have been found capable of producing skin cancer in mice. Tumors have been produced in some rabbits but they require a considerably longer time than in mice. Six rhesus monkeys have been tested with a fraction which is highly active, in so far as the production of cancer in mice is concerned, and in three years, all developed papillomas, but none has developed carcinoma. Only one rat has produced a tumor in fifty-five treated with fractions from the catalytic process, and no guinea pigs have been found susceptible.

No malignant tumors have occurred in men as a result of exposure to fractions from the catalytic process. The petroleum industry is well aware of the potentialities of some of the fractions in so far as mice are concerned, and careful surveys have been made of large numbers of men who have been exposed. Whether man is wholly resistant to the development of cancer from exposure to these oils, as the guinea pig seems to be, is yet to be determined. Meanwhile, extensive precautions have been and are being taken in some establishments of the petroleum industry to obviate any exposure that could be dangerous.

The activity of all fractions is not the same. Some of the fractions of high molecular weight are highly potent, some are slightly potent, and some do not produce tumors at all. As yet no single specific carcinogenic agent in the catalytic-cracked fractions has been identified, but it is known that the process does produce some

polycyclic compounds related to known carcinogenic hydrocarbons.

It has been found that dilution of the carcinogenic fractions with inactive components reduces the potency. Thus, when a mixture containing 55 per cent of the active fraction and 45 per cent of an innocuous fraction was tested, the carcinogenic activity was found to be diminished. If the amount of active fraction is only 29 per cent of the blend, no cancers will be produced although some papillomas will occur in mice. When only 10 per cent of the fraction boiling above 700° is present, the material has no carcinogenic activity.

The representative of one company stated that control measures that have been instituted are both medical and technical in nature. Medical measures include periodic examinations. No cancers have been found that could have resulted from these oils. Should any employee be found to have a suspicious lesion, he will immediately be transferred to a position where no further contact with the oil could occur. Educational programs have been instituted. Cleanliness is encouraged, and the men who come into intimate contact with the oil are urged to remove it promptly and to shower daily. The men have been told that the material causes tumors in mice and conceivably could cause tumors in men, and they are urged to be careful in working with the material and to report any skin lesion immediately.

The technical aspects of control include the handling of the oil in such a way that as few people as is absolutely necessary are exposed. All equipment that contains carcinogenic fractions carries labels indicating that the contents may be injurious and that should contamination occur, the oil must be immediately washed off. Men who must come into contact with active fractions are provided with protective clothing. Any fraction that is active is diluted to 10 per cent concentration with inactive fractions before leaving the plants and the mixtures are sold only for use as industrial fuels. Petroleum chemists are working to concentrate all carcinogens into a small fraction so that it can be disposed of safely.

The evidence that carcinogens may be produced by the catalytic process is this: when the material introduced for cracking is of

high molecular weight some low molecular-weight fractions are produced and some fractions comprised of molecules larger than any that were introduced into the cracker. Some of the latter may be carcinogenic.

The problem of carcinogenic agents in the petroleum industry is not limited to catalytic-cracked fractions. Some thermal-cracked, high boiling-point fractions are known to be carcinogenic, and indeed, some high boiling-point fractions from certain crude petroleums are also carcinogenic. Cancers of the scrotum have been observed, both here and abroad, in petroleum workers who were exposed to wax derivatives of certain types of crude oils. There have been about 1900 skin cancers in Great Britain in certain industries, in which there is exposure to processed shale oils. The occurrence of skin cancers in briquette workers has occurred in this country. A total of about 1700 to 1800 skin cancers from tar or pitch have been reported from various countries.

Arsenic in the form of Fowler's solution has caused carcinoma of the skin in many persons (145) who have taken the solution over a period of years for chronic skin disease. There have been some thirty cases of carcinoma of the skin from industrial exposure to arsenicals; contact with arsenic dust in sheep-dip workers is said to have caused cancer of the skin and lungs. The widespread use of arsenic-containing insecticides is a potential danger yet to be evaluated.

Radiation Carcinoma. Bloch has produced carcinoma of the skin in rabbits with soft roentgen rays. Cutaneous cancers from roentgen rays have occurred in many of the older radiologists as an occupational disease. Skin tumors in rats have been induced with beta rays.

Ionizing radiation, applied in low doses to the whole body over a long period of time, will cause earlier development and increased incidence of spontaneous tumors in experimental animals in some strains that are susceptible to such tumors. In mice, which are susceptible to leukemia, the disease occurs earlier in those that have received radiation than in those not irradiated. Other species tested do not develop leukemia more quickly after irradiation, even though they be susceptible to this form of neoplasm.

Pulmonary Cancer

The work of Machle and Gregorius was discussed and there was general agreement that the increased incidence of carcinoma in the lung in chromate workers is an indictment of *chromium*. There was a feeling that any hexavalent chromium compound might be carcinogenic. Experimental studies on animals, to test this possibility, are being conducted at Johns Hopkins University. A total of more than one hundred cases of cancer of the lung in chromate workers are now on record from American and German sources.

The possibility that *asbestos* might cause cancer of the lung was considered. The only evidence in favor of this was the fact that fourteen cases of carcinoma of the lung have occurred in ninety-two cases of asbestosis which have come to autopsy.

The clinical experience of Lanza and the experimental work by Vorwald have not revealed any carcinogenic properties which can be attributed to asbestos.

It was stated that in nickel refineries using the Mond process, there is a high incidence of carcinoma of the paranasal sinuses and of the lung. It is believed that this is due to the inhalation of vapors of *nickel carbonyl*.

Suspected or Potential Occupational Agents

The first evidence of *nuclear energy* causing occupational cancer in the United States was obtained by Martland, who proved the relation of osteosarcomas in radium watch-dial painters to their employment. Some twenty years later, this experience led to extreme care in the handling of artificial radioactive isotopes produced by cyclotrons and uranium piles. The carcinogenic potentialities of radioactive elements have been well demonstrated by animal experiments, but it must be admitted that the time is too short for us to draw any conclusions about their effects on man. It is true that the radioactive nature of these dangerous substances permits their ready detection so that proper monitoring should prevent dangerous exposures.

All isotopes that are distributed for commercial purposes by the

Atomic Energy Commission are sent only to people who understand the proper methods for handling them.

One potential source of overexposure to radiation now being investigated is found in certain textile and printing plants. There is a danger of fire starting in such plants as the sheets of cloth or paper move through them, due to the possibility of sparks resulting from static electricity. Bars containing radium are placed strategically near the cloth or paper in order to prevent the accumulation of dangerous electrical charges. There may be a real problem in potential cancer where many of these bars are used, or where they are improperly arranged or handled. This is currently being investigated in the State of New York.

Beryllium has become, during the last ten years, an increasingly important commercial substance and is now used in the manufacture of roentgen-ray apparatus, fluorescent lamps, certain types of radio tubes, and as an important source of neutrons in cyclotrons.

Beryllium is believed to cause two clinical types of pulmonary disease in people who have been exposed to fumes or dusts of its compounds. These consist of acute pneumonitis and chronic pulmonary fibrosis. In addition, the late Dr. L. U. Gardner was able to induce tumors of bone in rabbits by the intravenous injection of certain insoluble compounds of beryllium.

The beryllium-induced tumors have proved to be transplantable to the eyes of guinea pigs, by the method of Greene.

So far, no tumors have developed in man as a result of the exposure to beryllium.

General Considerations and Definitions

The Panel on the Epidemiology of Cancer approached the problem from the point of view of studies of the population as a whole or of selected groups. A concept of the epidemiology of cancer was developed and defined as embracing any and all information that describes the person or group of persons with cancer as compared with the control individual or group of individuals.

It is apparent that such information includes: (1) data on the etiological aspects, i.e., the extent of causative factors and the relationships between cancer and other pathological conditions in-

cluding the so-called "precancerous" lesions; (2) data describing the trends of cancer morbidity and mortality in a given population over a considerable period of time; and (3) control data to be used as indexes of changes in the cancer picture as related to the population. It was considered within the province of this panel to look into the sources and availability of such data with the aim of developing methods for the study and co-ordination of the epidemiological aspects of cancer in relation to existing bodies of clinical and experimental knowledge.

Sources of Information

Two general types of data were considered appropriate for studying the epidemiological aspects of cancer; namely, bodies of fixed, existing materials, i.e., set systems of records; and data derived from special field studies.

Existing Bodies of Set Data

Under the heading of existing records available for study, the following sources of information were discussed:

Health Department Records. State Health Departments have large existing bodies of data of four specific sorts:

1. Case Records from Cancer Registries
   These records can be used to initiate studies of the life experience of cancer patients. They form the starting point for follow-up, and the collection of analytical data relating not only to the cancer patient but to his family and relatives as well.
2. Industrial Health Reports
   Records of this sort could be used for studies of occupational and environmental factors, as related to the total cancer picture in an entire state.
3. Illness Data
   From morbidity records compiled in a chronic disease study, such as that reported from California, and from reports of absenteeism in industry, the frequency and disability from cancer can be studied.
4. Death Certificates
   Recorded deaths can provide leads in studying the epidemiological aspects of cancer. Death rates by site, occupation, etc.,

and studies in cancer mortality trends are possible through a fuller use of death certificates.

Since the State Health Department maintains a stable point of continuity relating to the public health of a relatively large population, it was considered to be the logical place for long-time studies in the epidemiology of cancer.

Life Insurance Material and Facilities. Although the records maintained by life insurance companies have been designed largely for actuarial purposes, they nevertheless yield certain by-products that could be used to advantage in developing new leads in the epidemiological approach to cancer.

Thus, death claim records for Ordinary, Industrial, and Group life insurance often lend themselves to the determination of cancer mortality rates for different sites—by age, race, sex, and state of residence. The death-claim records for Group life insurance may permit the determination of cancer death rates for employees in specific industries.

The medicoactuarial records maintained by many large life insurance companies for special studies (made periodically by the Joint Committee on Mortality of the Actuarial Society of America and the Association of Life Insurance Medical Directors) can be used to calculate cancer mortality rates for persons insured under Ordinary policies with different characteristics, such as a personal history of cancer, family history of cancer, different occupations, abnormal build, and various medical impairments. Cancer mortality rates so derived for persons with particular characteristics can be compared with the average cancer death rates among all insured lives.

Disability claim records available in many life insurance companies for individuals claiming disability because of cancer could be used to determine not only survival rates but also recovery rates and rates of recurrence of cancer after recovery. Similar use could probably be made of the hospitalization and surgical claim records.

It is believed that life insurance companies and the actuarial profession would be willing to co-operate in investigations of the

epidemiological aspects of cancer sponsored by the American Cancer Society. The agencies to approach are the Committee on Mortality and the Committee on Group Mortality and Morbidity of the Society of Actuaries.

Besides being an important source of information regarding cancer death rates, some of the large life insurance companies have a wide range of facilities for medical examinations; such facilities offer an opportunity for experimenting with simple tests for cancer. Life insurance companies may also be consulted in following up those cancer patients who carry life insurance.

Records on Military Personnel and Veterans. It was felt that the military and veterans populations were of sufficient size and importance to lend themselves to studies in the epidemiology of cancer. The nature of these populations is such that their characteristics are known, they are relatively easy to follow, and they could provide a useful body of data for study over a considerable period of time. Information from these sources would, of course, be limited in so far as occupational characteristics are concerned, but the age and the medical characteristics, as well as the follow-up information, could easily be obtained for study purposes. Again it was found that the agencies responsible for these sources of data would be willing to co-operate in any research studies that fell within the administrative framework of their activities.

Vital Statistics of the United States. Analyses of the Vital Statistics Records of the United States (which are limited primarily to population and mortality data) have already pointed up certain time trends relative to deaths from cancer of specific sites, as well as considerable geographical variations in reported deaths from cancer of various sites, as presented in the syllabus entitled "Selected Facts and Figures on Cancer" prepared by the Statistical Research Section of the American Cancer Society specifically for this Conference. It is believed that these materials (inadequate as they may be with respect to definitive diagnoses as to the cause of death) can be used to good purpose in developing leads into areas of fruitful investigation relative to the epidemiology of cancer. The subject of cancer mortality statistics according to occupation and industry for the United States, such as those developed by the

English, was discussed, and the need for such data was brought out.

Hospital Records. Hospital records have always been an excellent source of information for morbidity studies, particularly from the standpoint of definitive diagnosis, therapy, follow-up, and subsequent medical history. The only point of emphasis here was that much more basic information could be obtained from hospital records by closer co-ordination between clinical and experimental data, and by the cross-checking of various hospital registries for associated pathology.

Medical Examination Records of Large Corporations and Industrial Concerns. The attention of the Panel was drawn to the fact that many large corporations and industrial concerns have an accumulation of records relative to physical examinations, both pre-employment and periodic examinations while employed. In view of the fact that these sources of information have already been tapped and are believed to be of use in building up knowledge of the natural development of cardiovascular-renal diseases, it was recommended that they be considered as possible sources of information concerning the epidemiology of cancer.

Cancer Detection Center Data. It was pointed out that data in this category fall into a somewhat intermediate class somewhere between the bodies of existing, fixed data, already described, and those developed from field surveys to be discussed in later sections of this report; for example, cancer detection centers are already in possession of extensive records of medical examinations of supposedly well people and, in some instances, these records now include a series of examinations on the same person over a considerable period of time. To this body of fixed data could be added other information of the type usually collected by field-survey methods; namely, by careful questionnaire or interview techniques at the time of examination, numerous factors relative to familial, dietary, smoking, occupational, or industrial characteristics could be elicited for correlation with the presence or absence of any malignant tumor. Since serial examinations are the goal in many instances, detection center records represent an excellent potential source of information, not only on the time lag between the onset of symptoms and the establishment of well-defined

tumors, but also on the relationships between other pathological conditions, including the so-called "precancerous" lesions, and the possible subsequent development of known cancers.

It was found that the detection centers also offer a unique opportunity for controlled field testing of any new screening devices that may be developed for the early diagnosis of cancer. Since many of these centers are now being conducted under the sponsorship of the American Cancer Society, it was felt that the co-operation of many of them could be obtained for the purpose of investigations in the epidemiological aspects of cancer.

Other Sources of Data. Discussions of other sources of material available for possible leads into the study of the epidemiological aspects of cancer included records of other morbidity surveys, i.e., mass chest roentgenograms for tuberculosis, State Employment and Personnel Departments, Prepayment Health Insurance Plans, the National Registry of Twins, Genealogical and Historical Societies, and the existing morbidity and mortality records on cancer in various foreign countries.

It was felt that leads developed from existing bodies of data might be useful not only in determining the approach to certain laboratory and clinical investigations, but also in pointing up the most fruitful avenues of approach in the collection of a second type of data—those developed by the field survey technique.

Data Derived from Special Field Studies

It was felt that by the careful analysis of existing bodies of data, and by the extension of the leads resulting therefrom into the realm of special field studies, valuable information of many kinds could be developed that would bring pressure to bear in the solution of the cancer problem. The types of studies discussed here were as follows:

The Questionnaire Technique to Extend the Usefulness of Existing Records. It was felt that concise, consistent, and persistent questioning relative to such characteristics as circumcision, the history of nursing (both as a child and as a mother), the use of tobacco and alcohol, family history of cancer and associated path-

ology, and exact occupation over the years, would yield important information, which, in conjunction with the bodies of clinical data now being assembled, could give valuable leads to the epidemiological approach to cancer.

Special Studies on Family Groups. The study of cancer in twins or siblings characterized by differences in place of residence, living, eating, drinking, smoking, and other personal habits, as well as differences in socio-economic status, occupation, and religious and cultural background, was given consideration as an avenue of worth-while endeavor. Family studies relative to the possible inheritance of cancer were also discussed. Emphasis was placed on the fact that in such field studies, many difficulties are encountered, not only in the assessment of diagnoses and causes of death but in the establishment of suitable control series.

Special Studies on Various Ethnic, Religious, and Socio-Economic Groups. Consideration was given to the study of various religious orders and national groups characterized by differences in dietary and other living habits, to determine the possible relationships between such habits and the incidence of cancer. The populations of various state institutions (prisons, reformatories, and mental hospitals) were considered as possible areas for epidemiological investigations. The difficulties here were indicated in terms of inadequacies in medical diagnosis, either because of the customs and mores of certain groups, or because of inadequacies in medical facilities available to others.

Special Long-Time Studies on a Selected, Complete Population Group. It was agreed that the true nature of cancer, as it affects a population, could be revealed only through a careful study of the family groups making up that population, from the standpoint of occupation, living habits, religious customs and mores, and numerous other factors. Also required would be the collection of data on cancer incidence over a long period of time; i.e., twenty to twenty-five years, and a complete follow-up on the entire population with periodic physical examinations over the same period of time. It is immediately apparent that such a study would be extremely costly, but it was felt that any amount of money invested in such a project would be well spent if it elicited important in-

formation (as it undoubtedly would) on the natural history of cancer in a human population.

Methods of Approach

Various methods of approach to the problem of further exploring present leads and the development of new leads in the epidemiological approach to cancer were considered, and several points of agreement were reached.

First, it was agreed that the life-table technique (i.e., the actual survivors compared with expected survivors) is essential in the consideration of laboratory experiments as well as in human studies in the field of cancer.

Secondly, the importance of co-ordination of laboratory and clinical research with epidemiological field studies was emphasized. It was apparent that both approaches to the advancing of our knowledge of cancer would be enriched if the two approached were related one to the other. The way in which field epidemiological studies might suggest fruitful laboratory or clinical studies was indicated and, conversely, the importance of conducting laboratory and clinical research in such a manner that it could be taken into the field for confirmation, was emphasized.

The need for collaboration between the epidemiologist and the person interested in clinical research led to a discussion of the shortage of individuals having specific interest and training in the methods of conducting epidemiological studies. This shortage of personnel in the field led to one of the formal resolutions of the Panel. This resolution, and two additional ones dealing specifically with methods of approach to problems relative to the epidemiological aspects of cancer are presented verbatim.

RESOLUTION I

In view of the complexity of the problem and methods of analysis of epidemiological factors in human cancer and the importance of following existing leads and observations in this field, the panel recommends to the American Cancer Society that it establish a committee or subcommittee to continue the survey of these factors which was begun at this Conference: to suggest desirable studies in the field; to provide consultation in the design and conduct of such investigations; and where possible, to arrange

for joint or co-operative projects along these lines. It is suggested that the committee have members representing all sections of the country, and national agencies including the Public Health Service and the Actuarial Society of America.

RESOLUTION II

The panel recommends the establishment of a long-time epidemiological study of cancer in a population group, with careful observations over a period of years for evaluation of the familial, dietary, environmental, occupational, and other factors considered as possibly responsible for the development of cancer. *The objectives should be carefully defined and specific questions should be asked.* The committee or subcommittee, recommended in the previous resolution, should be utilized for the guidance of this research program.

RESOLUTION III

The panel recommends that the attention of the American Epidemiological Society be called to the relative lack of sound epidemiological knowledge of cancer, and that its co-operation be elicited in stimulating the application of present methods, and the development of more adequate methods in acquiring such knowledge.

PANEL ON PROFESSIONAL EDUCATION TECHNIQUES AS THEY RELATE TO CANCER

Moderators, FRANK E. ADAIR, M.D.
Cornell University Medical College and Memorial Hospital, New York, New York
OWEN H. WANGENSTEEN, M.D.
University of Minnesota, Minneapolis, Minnesota
Recorders, THOMAS F. ALMY, M.D., AND DAVID STATE, M.D.

TEACHING OF CANCER TO SCHOOL AND COLLEGE STUDENTS

Many efforts are being made to bring knowledge of cancer to the public by instruction in biology and health in schools and colleges. This has the advantage that all of the population is reached at an early age and, as some suggested, that parents are educated through their children. Additional gains are expected in that the individual will learn his responsibility, as a citizen of a democracy, to guard his own health and that of his family, and to support research and public-health activities related to cancer. It is further expected that the development of future scientists for the field of cancer research will be accelerated.

Those interested in cancer are fostering the better and earlier teaching of general biology to school children. This is being begun as early as the second grade. It is suggested that the problems of instruction at this level be met by explaining biological facts in terms of familiar analogies and by consultation with experienced writers of books for small children. More elaborate teaching of biology is done at the high-school level, and information related to cancer is fitted into programs that include personal hygiene, mental hygiene, family living, disease, and disease prevention. It was pointed out that the citizen of the future needs to develop a concept of growth and disease including not only states of acute illness and debilitation but also the more subtle bodily changes of insidious disease. The teaching of science can well stand as a cultural subject, and it should replace certain traditional but outdated subjects such as ancient languages and military history. Demon-

strations such as are provided by the Cleveland Health Museum are valuable aids to the teaching of school children.

For many years, college students have been invited to carry on research work at the Jackson Memorial Laboratory. Because of the intensity of their interest, this program has more recently been extended to include high-school students during their summer vacation. Since these positions are filled on a competitive basis, it is expected that the interest of large numbers of potential scientists will be stimulated.

Teaching directly related to the cancer problem should be given in the schools, but there was a difference of opinion as to whether this should be begun in elementary schools. There was greater agreement that the teaching of cancer should be introduced at the secondary school level. Many schools now include such teaching in biology, mathematics (statistics), history (e.g., development of surgical and radiation therapy), and social sciences (statistics, social and economic problems of degenerative disease). A variety of methods is used. Special interest and some controversy attaches to the techniques of demonstration of the methods of self-examination, such as a movie used in Idaho to teach high-school girls to examine their own breasts.

Reference was made to a policy statement on the teaching of cancer in elementary and secondary schools, prepared by an advisory committee to the American Cancer Society (A Statement of Principles as a Guide for Cancer Education in the Schools. New York American Cancer Society, March, 1949). This committee was composed of representatives from the fields of education and public health, at national, state, and local levels, and from the Divisions of the American Cancer Society. It is expected that the fear of cancer will be reduced rather than increased by early education, and that an attitude of wise caution will be substituted. With an increasing awareness of cancer in the general population, physicians will have to abandon the practice of trying to conceal the diagnosis of cancer from the patient.

The aid of governmental and voluntary agencies would be useful in providing source information on scientifically correct data, in making available visual aids, and in providing medical consultants.

Training of School Teachers in Cancer Teaching

Biology teachers in the schools often require assistance in giving effective instruction in cancer to their pupils. Because cancer is widely regarded as a process of "abnormal" growth, it is not properly covered in textbooks in general biology. With present emphasis upon the social sciences, special efforts to impress teachers with the importance of biological science are necessary. In the institutions for training of teachers, this is being done by courses in adult personal hygiene, and by didactic, laboratory, and field work on public-health problems, social problems related to disease, and community health resources. There is need for the postgraduate education of teachers, and this is being done by the distribution of manuals on cancer education and by visits to health museums. Refresher courses would be a valuable aid, and it is suggested that the arrangement of these would be a proper function of state education programs.

The Public-Health Educator in Relation to Cancer Teaching

Research in sociology, anthropology, and psychiatry has indicated the need for breaking down cultural barriers to the protection of personal health. The public-health educator serves as liaison between the public, the volunteer health workers, and the physicians, by locating the leaders of cultural groups and finding ways of influencing the attitudes and behavior of persons in these groups toward the diagnosis of serious disease. Such groups are stimulated to discuss health problems, and to take whatever action is necessary in their communities to improve health conditions. The training of the public-health educator should include many of the disciplines of public-health schools, plus familiarity with educational techniques and with the particular problems of the diseases with which she concerns herself. The American Cancer Society is already providing funds to assist in training of this sort.

Special Training of the Nurse for Her Role in the Cancer Problem

The nurse is conceived as a member of a team devoted to the solution of the problem of the cancer patient. This includes the

patient himself, the nurse, the physician, the medical social worker, and the various specialists in the field of rehabilitation. To fit the nurse better for her role in this work, postgraduate courses have been established in at least two universities. These are taught chiefly by physicians and nurse educators and cover both personal and community aspects of illness due to cancer.

Undergraduate Teaching of Cancer in the Medical School

About ten years ago, methods for the improvement of cancer teaching in the medical schools came under the consideration of the National Advisory Cancer Council. Within the past two years, a program of this sort has been rapidly developed to include nearly every medical and dental school in the United States. Each medical school receives assistance from the National Cancer Institute in an amount of approximately $25,000 per year, and each dental school receives about $5,000. In the last year, these programs have been organized in each school under the direction of a "cancer co-ordinator," and these men have met to exchange ideas on methods of improvement in cancer teaching.

A number of common problems have been encountered in the conduct of these teaching projects. The concept of the teaching of cancer as a unified subject has at first seemed to conflict with orthodox principles of "horizontal teaching"; this is made more serious because of the inflexible nature of the medical curriculum. In many instances, poor organization of clinical teaching has needed correction, and follow-up facilities have been found inadequate.

It was early recognized that because of differences in the structure of the various medical schools, many special problems exist, and that no standard formula would be found for their solution. In most instances, however, committees for the study of local problems have been set up and better co-ordinated teaching has begun to appear.

Wangensteen suggested that, since the purpose of the Panel was to discuss the problem of the teaching of cancer as it exists in our teaching institutions, it might be well to reverse the order of stating objectives first and then discussing them, by discussing the topic first and then stipulating certain desirable objectives in the

problem of cancer teaching. He pointed out that, at first, surgeons and pathologists were primarily interested in the problem, but that now many new branches of medicine have been included.

Ravdin suggested that teaching undergraduate medical students is the so-called "grass roots" level. The value of the teaching to the undergraduate students is governed not only by the type of teacher but also by the accuracy of the teaching. It is still common, Ravdin noted, to find that rectal examinations are being omitted from routine examinations, yet 50 per cent of the carcinomas of the large bowel are found in the rectum. Gastric ulcers are also being treated medically and yet there is a high degree of error in differentiating benignancy from malignancy in this condition.

Arthur Purdy Stout indicated that the teaching at the undergraduate level should consist essentially of training in what carcinoma is, how it may be best treated; in fact, emphasis should be placed upon the fact that carcinoma is not an incurable disease, since far too many students leave the medical school at the present time with the impression that cancer is a hopeless condition. He felt that the cancer co-ordinating program was of definite value in this respect. At the graduate teaching level, as it pertains to pathology, he has had a number of surgeons working in the pathology of carcinoma before entering the specialized field of carcinoma therapy. In addition, local groups of pathologists have staged seminars, directed by experts in this field as moderators, to discuss the pathological and clinical features of cancer. These have been of great value in improving the knowledge and understanding of cancer diagnostic problems by general pathologists. He found that the value of cancer teaching groups, if well organized, was definitely good, but where the instruction was only for two to three days at a time, the results were of questionable value.

At the University of Minnesota, correlation clinics are conducted during the courses in anatomy and pathology. A period in the tumor clinic and tumor conferences are provided in the third year, and a didactic "vertical" course in cancer is given to the seniors.

At Yale, the contact of the student with clinical material has been increased and his view of the cancer problem has been ex-

tended to include the social and psychological problems of the cancer patient. The over-all object is to promote in the student the ability to continue to educate himself, and the wisdom to see the need for it.

At New York University, didactic lectures in cancer have been greatly improved, and a teaching program has been set up for their dental school. A cancer clinic and a detection clinic have been established. Much valuable information has been gained from a questionnaire given to third- and fourth-year students. This revealed not only individual variations in knowledge but also general gaps in knowledge attributable to inadequate teaching.

The importance of research to the improvement of cancer teaching is illustrated by the program at the University of Kansas. Medical students, fellows, house staff, faculty, and undergraduate college students are encouraged and aided in performing or participating in research projects during their summer or elective periods or other free time. Further stimulus and interest is provided by the presentation of such research work at meetings attended by students and staff, and research prizes are awarded. Cancer research is of value not only for the results per se but to stimulate interest in cancer, to increase knowledge about cancer, and for a more proper understanding of cancer as a living, vital biological problem.

At the University of Colorado, great advantage has been gained from an early and clear understanding of the relationship between the cancer project and the existing departments. The responsibility for the care of patients with tumors of specific anatomical sites has been carefully assigned, and the various departments have been given fixed responsibilities in the conduct of the tumor clinic.

At the University of Southern California, a course in fundamental concepts of cancer has been introduced into the third-year teaching. In the senior year, the teaching of clinical cancer has been improved without disturbing the existing curriculum, by the symposium method, which brings together representatives of various departments.

At Tufts, the effort in cancer teaching has been centered about the cancer research and control unit. Investigations on cancer pa-

tients have been favored by affiliations with hospitals for terminal care, in which new research laboratories have been established. A concentrated course in cancer teaching is given in the fourth year.

At Cornell, emphasis has been laid upon interdepartmental teaching, utilizing the techniques of the symposium and the panel discussion. These are applied to a review of important tumors for the fourth-year student and the house officer. This method of teaching has favored the interchange of ideas among members of the teaching staff, and is expected to pave the way for future innovations in cancer teaching.

At the Ohio State University College of Dentistry, the co-ordinated teaching of cancer has been carried on in preclinical and clinical departments. No separate course in cancer is offered. Introduction of a cancer-research project has served to stimulate the interest of the various departments.

Bierman presented an analysis of the educational levels of the students at various stages of their medical education at the University of California Medical School, as ascertained by a series of objective multiple questions. It was found that the level of knowledge of the students in the field of cancer progressed from the freshman to the senior year, and in addition, the interns had a greater knowledge of the over-all field than did the senior medical students. Interestingly enough, the teaching staff, however, did not possess as great an over-all knowledge of the field of cancer as did the senior medical students or the interns. He felt this was due primarily to the specialized interests of the staff who had relatively little knowledge of cancer as it pertained to other specialized fields. He pointed out that the amount of material that the medical student must learn today has increased greatly, yet the time allotted for such teaching has not altered appreciably. In an effort to meet this problem the following alternatives in the alteration of the medical-school curriculum were suggested:

1. Expansion or prolongation of the medical course.
2. Earlier specialization with the medical school.
3. The omission of the less essential information in medical teaching.

4. The better integration and streamlining of the medical program.

Robert Moore felt that it is not possible to lengthen the students' curriculum, but he did feel that some courses in the medical curriculum could well be eliminated. The better integration of the teaching program across departmental lines was probably the best solution to the problem.

In the last analysis, it was emphasized, the teaching of cancer must be made adaptable to the purposes of the student entering the fields of practice, teaching, and research. The enthusiasm of the teacher is the most important factor in good teaching. There is need to develop the interest of specialists in internal medicine in clinical investigations on cancer problems.

The intensified consideration of a special subject at all levels of the medical curriculum should not, and probably will not, be confined to the field of cancer. "Vertical Teaching" may need to be limited since it encourages education by rote instead of by principle.

Fellowship Training

Spencer pointed out that the health educator brought the experts in the field of cancer and the lay public closer together. His functions were not those of teaching; rather, they were the administration in the field of teaching. In 1938, the National Cancer Institute made its first fellowship appointment. By February 15, 1949, 163 trainees had completed training at various approved institutions. Such training has resulted in an increased quality of patient care, earlier diagnosis, and better treatment. This activity has been one of the most popular of the National Cancer Institute. He stated that there have been no attempts made to train a man in all the aspects of cancer treatment in any particular instance; the fellowships have been awarded to men in the various specialties in the treatment of cancer, such as surgery and radiotherapy.

The basic minimum requirements for fellowships are as follows:

1. The applicant must be graduated from a grade-A medical school.

2. He must be less than forty years of age.
3. He must have had a one-year rotating internship.
4. He must have had an approved residency.
5. He must have the approval of the institution before receiving the fellowship.

It was expected that 50 per cent of the time of the fellow would be devoted to cancer diagnosis and therapy.

Meader added that these fellowships are at three levels:

1. Postdoctoral.
2. For students preparing themselves for the Master of Science degree.
3. In a few instances, for students with a Bachelor of Science degree, working on a special program.

As a result of a questionnaire sent to 111 trainees who had completed their training, the following suggestions were offered to improve the fellowships:

1. The surgeons from whom training is to be obtained should have more knowledge of the pathology and the radiotherapy of cancer.
2. The trainees should have more extensive training in surgery prior to fellowship.
3. Part of the training should be devoted to cancer research.
4. More instructions in the care of terminal cases should be included.
5. Fellowships should be given to centers of learning that are more active in the teaching and care of cancer.
6. Certificates should be given to each trainee at the completion of the fellowship.

There are now 140 active fellows in the National Cancer Institute Fellowship Program, and $500,000 has been set aside for the financing of the fellowship program for the coming year.

Like the fellowships under the Committee on Growth Program, those under the National Cancer Institute are tax exempt.

Teeter stated that the total cancer program through the Ameri-

can Cancer Society, the National Cancer Institute, The Damon Runyon Fund, and The Atomic Energy Commission, has resulted in the expenditure of $80,000,000 in ten years; $60,000,000 was spent in the last two years of which $20,000,000 has been expended for research. The Damon Runyon Fund has been working in close co-operation with the National Cancer Institute, fellowships being awarded through the joint action of the American Cancer Society and the Committee on Growth. For the coming year, $150,000 has been set aside to permit the training of forty fellows. Teeter emphasized that the Damon Runyon Fellowship Fund will be expended principally in the field of applied or developmental research. He also felt that money should be made available for the so-called career scientists; that is, support should be found for promising scientists so that they may remain in the field of research.

Lehman also pointed out that the American Cancer Society is offering fellowships of the same type as the National Cancer Institute with criteria for fellowships much the same as for the National Cancer Institute but that there was still the problem of the criteria for an institution to which these fellowships should be awarded.

Brewster Miller stated that in 1947-1948, the American Cancer Society allotted twenty cancer fellowships. In 1949-1950, twenty more or a total of forty will be available. Miller asked the Panel to comment on the possibilities of setting up criteria that might pertain to the institutions and doctors eligible for such fellowships. He indicated that the institution makes the choice of a fellow to be appointed. Although there was some discussion of eligible institutions, no definite recommendations were made. It was felt that no rigid rules should be laid down but rather the merits of the individual and the institution should be given full consideration in each particular case. There was considerable debate on the relative merits of teaching fellows at special cancer hospitals and large general hospitals or university centers.

Nicholson stressed the part played by the Committee on Growth in relation to fellowship training. This committee deals with the problem of growth, both abnormal and normal, in the broad aspect. The objectives of the fellowship training program is the

training of personnel to become cancer investigators. In the fellowship qualifications reviewed by the Committee, the following qualifications are stressed:

1. The applicant's ability and promise as an investigator.
2. His interest or potential interest in the problem of cancer.

The fellowship deals primarily with, or stresses primarily, the training of an individual rather than the staffing or the particular pursuit of a problem. The number of appointees at the present time under supervision of the Committee on Growth is fifty.

Postgraduate Training

The General Practitioner. In the field of postgraduate education as it pertains to the general practitioner, Allan Ryan referred to the fact that the cancer diagnostic clinic to which the practitioner brings his patient to be seen and studied by cancer specialists has improved the diagnosis and treatment of cancer. In most instances, these diagnostic clinics have been held in association with tumor clinics at general hospitals.

Popma felt that there was a need to survey the smaller communities where there are no specialists within the field of cancer diagnosis and cancer therapy.

Neff stressed the value of postgraduate teaching through the Texas Cancer Bulletin, which makes an effort to bring to the general practitioner the latest facts concerning the diagnosis and treatment of cancer.

Pack felt that in the postgraduate education of a general practitioner, the emphasis should be upon early diagnosis of cancer. In the survey of culpability for delay, made at Memorial Hospital ten years ago, two thirds of the fault was due to the patient, one third of the fault due to the doctor. A similar survey, carried out ten years later, shows that there is still considerable delay on the part of physicians in making the diagnosis of cancer. He felt there was a real place for the so-called short or refresher courses for practicing physicians, which lasted from one to three months during which time diagnosis and therapy of cancer were stressed.

Wangensteen suggested that one of the real services that could be rendered to the general practitioner is to publicize the fact that there are a great number of cures in patients who have carcinoma. He recommended that the American Cancer Society make available, by publishing data on this information, the fact that the curability of cancer is great and can be still further improved by the early diagnosis of cancer.

At the University of Pennsylvania, Ravdin reported, a graduate teaching program has been set up for the general practitioner, in which the participants stay at the University for two weeks at a time, living quite closely with the teaching personnel. Methods of treatment that are sound and yet not too controversial are recommended. Discussions and teaching are carried out primarily in the form of symposia, except in the teaching of the fundamental biological sciences in which formal lectures are given. It was felt that this form of refresher course has been most valuable in the effort to cover the gap of continued education on the part of the practitioner after he leaves the medical school.

Heacock outlined the teaching program for general practitioners in the State of Tennessee. The state it divided into ten circuits, each so placed that every general practitioner is within twenty-five miles' distance of a teaching unit. The unit consists of a field director and an instructor whose purpose is to teach the general practitioner the various aspects of the early diagnosis of cancer and its treatment as well as to act as a consultant in the handling of individual cases. These units and the teaching program are supported jointly by the Tennessee State Medical Society, The Tennessee Division of the American Cancer Society, the Department of Health in the State of Tennessee, and The University of Tennessee Medical School. The enrollment and attendance have been high at each of these instructional periods. In addition to the teaching program, there have been an increasing number of cancer diagnostic clinics with four additionally approved clinics for cancer therapy.

Lehman pointed out that in teaching the practitioner, the matter of alertness to the problem of cancer was to be emphasized, because in many cases it was not a matter of ignorance on the part

of the doctor but rather a lack of alertness to the possibility of carcinoma that resulted in the late diagnosis of cancer.

The Resident. In teaching at the resident level, Ravdin stated that it was important to emphasize that before training in the specialized field of cancer was begun, it was essential to have a broad fundamental learning in the field of medicine.

Lehman agreed essentially with Ravdin's viewpoint on the matter of resident training. He too stated that before specialized training in cancer is to be embarked upon, the physician should have a broad knowledge of the field of medicine. He felt that the teaching of cancer could be best accomplished by specialists within limited fields.

A greater stress should be placed on the importance of endocrinology in the training program of residents according to Twombly. A better knowledge of the physiology of the endocrine organs as well as the clinical picture observed when abnormal alterations of the endocrine glands are present should be developed in a teaching program. More information also should be made available concerning the control of cancerous growths or amelioration of symptoms by the use of various types of hormones.

Lazlo made a plea for a more active participation of the internists in the teaching and handling of cancerous conditions. He felt that the early diagnosis of carcinoma is within the field of interest of the internist and that medical residents and interns should receive more instruction in the problem of the early diagnosis of carcinoma.

Wangensteen felt that a great improvement in the over-all picture of the end results of carcinoma therapy could be obtained by the earlier diagnosis of cancer by the internist as well as by the general practitioner.

Rawson stated that not only should the internist play a part in the early diagnosis of carcinoma but that the medical man should have a definite place in the pre- and postoperative care of the patient with carcinoma. He pointed out that in large institutions, 65 per cent of the patients who had one or another type of carcinoma had medical conditions requiring medical attention. He felt that a team consisting of a surgeon, a pathologist, a radiotherapist,

and an internist should participate in the handling of the patient with carcinoma.

The teaching of cancer in the vertical direction, that is, teaching of cancer per se was of no value to the student but might be of some value to the practitioner according to Newell. He felt that "oncology" was a poor specialty in medicine; "oncologist" could be better applied to the person in the research field of cancer. The physician who professes to teach cancer should have a background in the field of medicine, for specialization in smaller fields tended to make for a lack of awareness of all the deviations in the body that may be present along with cancer. Similarly, in therapy, unless there is interchange of ideas amongst the specialists interested in many aspects of cancer, there may be a tendency for a tubular vision on the part of one individual in charge of the treatment. The teaching of the medical students, he indicated, was best done by a number of men interested in the field of cancer but instructions on the part of each limited to his particular special field.

Fred Stewart said that the term "oncologist" did not mean a great deal to him and a physician hoping to have special training in the field of cancer should have a previous general surgical residency of three- or four-years' duration. Once formal training in cancer was started, he stated that broad contact with all major neoplastic diseases was important; this could perhaps best be obtained in a special cancer hospital.

Ackerman pointed out the value of a clinical pathologist who presented a dynamic view of the evolution of the disease and correlated the clinical history with the pathology of the condition. Such a person should work in close co-operation with the surgeon and radiotherapist. The pathologist should also train surgeons and radiotherapists to the value and limitations of the study of pathology. In addition, clinical pathological conferences with a pathologist interested in the life history of this disease would be of great value to physicians who were being trained in cancer therapy.

Regato emphasized that there are only twenty-nine full-time radiotherapists but that there were many other men who are doing radiotherapy in addition to diagnostic radiology. He believed it

would be advantageous to have more physicians trained in radiotherapy.

Finesinger discussed the part of the psychiatrist in the field of cancer teaching. The patient should be treated as a unit or a whole rather than emphasis placed upon the disease, such as cancer, within the confines of the body. The total disability that might accrue from any disease might be influenced greatly by the personality of the individual and his ability to handle a given crisis. In outlining a teaching program, Finesinger felt that a broad concept of the disease and its relationship to the personality of the patient as well as the means or manners of developing the appropriate doctor-patient relationship should be stressed.

Summary

The levels at which the problem of teaching cancer were discussed were: the medical-student, the fellowship, and the practitioner.

Student Level. The importance of adequate teaching of cancer at this grass-roots level was emphasized. Although various descriptive adjectives such as vertical, horizontal, diagonal, aerial, have been applied to the manner of teaching and the relative merits of each of these systems discussed, still it was the consensus of the group that the most important influence in the teaching of these students was the type, quality, and accuracy of the instructors. It was felt that the teaching of cancer could best be done by specialists who give instructions within their specialized fields, but that a definite integration of the various departments to emphasize the over-all broad picture of cancer should be carried on. The cancer co-ordinator program has done a great deal to bring about this integration of the teaching program.

An attempt to evaluate objectively the efficacy of the teaching of cancer by series of multiple questions was presented. From this study it was obvious that although the medical school curricula had changed little in the last twenty years, the amount of material that the individual student is asked to assimilate has increased tremendously. The suggestions offered to meet this acute problem were: (1) increase in the length of time of the medical school cur-

ricula; (2) omission of the less essential courses; (3) earlier specialization in the medical school for an integration and streamlining of the present mode of teaching. No definite recommendations, however, were made by the Panel regarding this problem.

Fellowship Level. It was the consensus of the group that the physician who intended to specialize in the field of cancer must bring to the problem a broad experience in the field of medicine before specialization is begun. The institutions that aim to provide postgraduate teaching in the field of cancer should have available a capable staff and a certain number of cases to permit the trainee to have a broad experience with the problem of cancer. It was felt that special cancer centers, university hospitals, general hospitals, and in certain select instances certain preceptors, could afford adequate forms of training. There was some discussion as to the best place to teach cancer at this level—in a general hospital or in a cancer hospital. While some Panel members felt that training in a cancer hospital should be, in effect, graduate training, there was no general agreement on this point nor on where cancer could best be taught. It was stressed that the institution or individual who was responsible for the teaching of fellows should provide not only the material for cancer instruction but also should give stimulus for thought in the problems of cancer to the trainee.

There have been an increasing number of fellowships made available through various agencies. The National Cancer Institute, American Cancer Society, Atomic Energy Commission, Committee on Growth, and the Damon Runyon Fellowship Fund, were amongst these. Eligibility of the applicants, the purposes and type of training, the eligible institutions for such fellowships were discussed. It was felt by all that the efforts of these agencies in training of individuals in the field of cancer have made and will continue to make an increasing impact on the early diagnosis and treatment and research aspects of cancer.

Some discussion was lent the problem of whether a fellow's training should be largely in one of the cancer fields such as surgery, pathology, or radiology, or whether he should be broadly trained as an oncologist, professing ultimately a certain degree of expertness in all these fields. The opinion on this matter was di-

vided and reflected largely the type of training to which each speaker had been exposed, or reflected his present attitude relative to his own breadth of competence in the field. In the main, it was largely agreed that it was unlikely, if not impossible, for a cancer trainee to acquire an equal degree of competence and facility in several branches of cancer management.

Instruction of the General Practitioner. The general practitioner continues to be the initial outpost in the discovery of cancer. It was felt that continued education of the practitioner was of the greatest importance. Various means of accomplishing this were described: (1) intensive two- to three-week programs at medical centers or approved cancer hospitals where the practicing physician lives in the institution, is given lectures, sees patients, and attends conferences; (2) cancer diagnostic clinics and tumor boards, where the doctor brings his patient to a clinic and the cases are discussed by the attending specialist with the physician: by this means, instruction in the early diagnosis and treatment of cancer are made available; (3) distribution of informative literature in the problem such as is being done in Texas through the Texas Cancer Bulletin; (4) the Tennessee program where an active program of teaching is carried out in a number of centers throughout the State of Tennessee. It was felt that the early diagnosis and the care of those with cancer should be emphasized in the instruction to the attending physician. The needs of smaller communities in sparsely populated portions of the country were specifically noted, but no practical solution to the problem was offered. It was hoped that the training of more physicians in the field of cancer diagnosis and treatment would make available a greater number of trained individuals for the smaller community in the country.

The Teaching Personnel. In the instruction of pathologists in the field of cancer, a suggestion was made that the clinical aspects of the disease and its relationship to the life history of the cancer should be stressed in the teaching of trainees in the field of pathology. There are probably only a very few individuals who have actual skill and experience in pathology, surgical technique, and practical knowledge of radiation therapy. However, there are a number of physicians who are specially skilled in one or more of

these branches and also possess understanding of the limits of the other branches. Improvement in the techniques of cancer treatment will come from such men or from groups of specially trained men working in close co-operation.

The need for more active participation on the part of the internists was emphasized. The field of early diagnosis and the care of patients both pre- and postoperatively were mentioned as specific fields in which the internist might participate more actively than he has in the past. The potential role of the psychiatrist in the care of cancer patients was discussed. It was felt a real contribution could be made not only in the teaching but also in the care of the patient, for he must be considered as an individual rather than as a harborer of an isolated lesion such as carcinoma.

PANEL ON SERVICES AND FACILITIES

Moderators, CHARLES F. BRANCH, M.D.
American College of Surgeons, Chicago, Illinois
EDWARD D. SUGARBAKER, M.D.
Sugarbaker Clinic, Jefferson City, Missouri
Recorder, THOMAS F. NEALON, M.D.

The Moderator briefly reviewed the development of the cancer-control program particularly as it related to cancer clinics, cancer diagnostic clinics, and cancer detection centers. When, in 1930, the American College of Surgeons, at the request of the Directors of the American Society for the Control of Cancer (now the American Cancer Society) formulated Minimum Standards for Cancer Clinics in general hospitals, only a small number of clinics established in these hospitals met the standards: 106, according to the approval list published in October, 1931. The last approval list, published in December, 1948, listed 530 cancer clinics. Although this has been a very marked increase, yet only approximately 25

per cent of the hospitals of 100 or more beds provide recognized cancer clinics. This is approximately the same ratio of clinics to hospitals that existed in 1931.

Past experience has shown that the presence of an approved clinic in a particular area has resulted in an improvement in diagnosis and treatment of cancer patients.

The December, 1948, issue of the Bulletin of the American College of Surgeons also carried the first list of cancer detection centers meeting the minimum requirements as set forth by the American College of Surgeons.

Cancer Hospitals

There are, at present, eleven cancer hospitals in this country, and eight additional ones are in the process of construction. These are being built at an average cost of $7,000,000 plus an additional $1,500,000 for equipment.

The feasibility of large cancer hospitals and particularly of one designed to investigate the possibilities of the products of radioactive fission in the treatment of cancer was discussed. The consensus of opinion was that a hospital constructed for the sole purpose of investigating the possibilities of isotopes in the treatment of cancer would be unjustified. However, a hospital, the major portion of which would be devoted to the general care of cancer patients, but in which an adequate portion would be used for the investigation of isotopes in cancer therapy, was considered worth while. Members of the staffs of different existing cancer hospitals stressed that their hospitals pointed the way to improving techniques in the diagnosis and treatment of cancer in the particular area in which they were situated and that such improvement had occurred. The hospital was also of value in the education of both the public and the profession in the area coming under its influence.

There was ready agreement that the care of the cancer patient in the cancer hospital is better than that given in the average general hospital, but that this does not hold true when the general hospital is connected with a teaching institution. One of the participants asked whether special hospitals for all diseases should be

considered. It was felt that the care of the cancer patient in the nation as a whole could be best improved by elevating the standard of care at the level of the general hospital. Since such care had been found to improve with the establishment of an approved cancer clinic in a hospital, it was felt that improvement on a national scale could be achieved by the establishment of cancer clinics in general hospitals.

The new clinical center under construction in connection with the National Cancer Institute was discussed. Diebert stated that this center was designed for basic and clinical research and not for general care of cancer patients.

Cancer Clinics

No cancer control officer present felt that adequate facilities now existed, in his state, for treating cancer patients in that state. Recommendations on how the facilities might be improved included:

1. Building more general hospitals.
2. Adding facilities in existing general hospitals.
3. Developing facilities for rural communities.
4. Attempting to have a cancer facility within a reasonable distance of each cancer patient or potential cancer patient.
5. Developing mobile units of diagnosis and treatment.
6. Developing mobile units of experts to conduct cancer programs and establish clinics.

On the question of the relationship between population and cancer clinics, it was felt that there should be a clinic:

1. For every 100,000 population.
2. Within a radius of 30 to 50 miles from the cancer patient. (Some consideration should be given here to the importance of distance in rural and in suburban areas.)

Discussion of the operating costs of cancer clinics brought out:

1. The initial cost of establishing a cancer clinic varied considerably in different areas (figures quoted varied from $3,000 to $30,000).

2. The initial cost is the greatest, by far. In a few instances clinics once established were able to maintain themselves.
3. In many areas, support is available from the American Cancer Society for both the establishment and maintenance of these clinics.

The discussion then turned to the question of how physicians trained in various specialties might be encouraged to settle in particular areas. An analysis of the Directory of the American Medical Association has shown that for every 100 physicians, there is one-half pathologist, three and one-half radiologists, and four and one-half surgeons. Unfortunately, this ratio is not typical of many areas. It appeared that areas of smaller population with insufficient medical specialists could probably best avail themselves of the necessary services by taking advantage of mobile units of such specialists.

The problem of how cancer clinics in particular areas might be set up with a minimum of the friction that has been attendant upon the establishment of the clinics in some areas was raised. A representative from a state that has been particularly successful in the development of a cancer-clinic program told how an active and competent cancer committee of the State Medical Society took the lead in the program and set the standards for it. The quality of the committee was well recognized by the local county units and recommendations from the committee were usually met with enthusiasm by the local county medical societies. In this program, there is no disturbance of the normal physician-patient relationship. It was felt that there was really no problem that could not be solved when physicians within a particular area saw eye to eye.

Cancer Diagnostic Centers

Branch said he had been most impressed by the fact that the majority of the presently approved cancer clinics came into existence as cancer diagnostic clinics. As participating physicians became more interested and gained confidence in the program, they worked together to get the necessary equipment to form a cancer clinic.

Cancer Detection Centers

A brief résumé of the development of the cancer detection program was admirably presented by MacFarlane, who felt that the experiments carried on by the pioneers of this field have proved the value of detection. She recommended that every State-financed hospital should have a detection center for those in the lower income brackets, but that the detection of cancer in individuals in the middle and upper income brackets be the responsibility of the individual physician. If each general practitioner would agree to do one detection examination per day it would go far toward supplying this need. Considering the fact that this examination would be limited to certain age categories, this suggestion would more than meet the need in New York City where there is one doctor to every 445 patients. The doctor would probably eventually receive dividends in time as a result of this program, since the detection of an early case of cancer and its subsequent cure would relieve the necessity of later having to devote considerable time to the care of a terminal cancer patient. She pointed out that the State of Pennsylvania is initiating a program under the direction of the individual County Medical Societies, whereby detection examinations will be carried on in doctors' offices.

She cited one example in which the American Cancer Society and the local tuberculosis units are co-operating to provide better case finding facilities for both cancer and tuberculosis patients.

Since the American Cancer Society has convinced the public of the worth of detection examinations and has its support, it was pointed out that the Society now has the responsibility of developing the method whereby the detection program can be carried out.

A report was made on a survey, carried out by the American Cancer Society, which included the experiences of 125 centers throughout the country and involved 51,728 examinations. Of the patients examined, 0.8 per cent were found to have proved cancer, 10 per cent had precancerous lesions, and 52 per cent were referred to private physicians or clinics for treatment of non-neoplastic diseases. The total number of cancers detected was 406. Several

recommendations were made on the basis of the experiences reflected in the survey.

Twenty-two cases of cancer were found in examinations involving 8897 men less than 50 years of age. Of these, nineteen might be classified as apparent (skin, lip, penis, tongue). Of the remaining three, two involved the intestinal tract and occurred in men in their late forties. It was felt that the best yield in cancer detected in men might be in those older than 45 years.

Six cases were found in examinations of 5339 women less than 30 years of age. Four of these were advanced cases and the stage of the other two was unknown. There was a definite increase in the incidence of cancer detected in women more than 30 years old, and this increase became more marked with each succeeding five years. On the basis of the material available, it would seem that in women, the examinations might be restricted to those 30 and more years of age.

A procedure was recommended for examinations carried on by the physician in his office that offers potentialities of detecting cancers in numbers closely approximating the results of the detection centers whose experiences were included in the survey. This would involve an examination of the oral cavity, skin, breast, pelvis, and rectum, plus a urinalysis and stool examination for occult blood. In addition, some arrangement might be made whereby the doctor could do a vaginal smear and send it to a central laboratory or a competent cytologist for interpretation; and another, whereby a roentgenogram of the chest might be taken, again either in a central laboratory or in the office of a participating radiologist.

This survey included most if not all of the well-known detection centers. It was suggested that the American Cancer Society should attempt to provide facilities for the examination of those individuals who could not afford to pay for this examination themselves and were not provided for in some other way—in other words, the medically indigent. Where funds are available, the Society should consider implementing the program so that the Papanicolaou smear would be available in the doctor's office and, in co-operation with the other agencies, both voluntary and official, an attempt be

made to make chest roentgenograms available. The program could well use a central office where records and information could be kept regarding the dates of follow-up examinations as well as on cancers detected. It would be the responsibility of this office to notify both the physician and patient when repeat examinations should be performed and indicate to the physician incidental pathology that might have been found at a previous examination.

Cameron pointed out that by limiting a detection examination to certain accessible sites with high incidences, the yields of cancers detected per unit of physicians' time would be markedly increased.

The Panel was given a report of the program of detection in operation in Hallsdale County, Michigan. In its first year of operation, 1248 patients, both with and without symptoms, submitted to a detection examination carried on by the physicians in their own offices. Thirty-five cases of cancer were detected. This represented 70 per cent of all cancer cases found in Hillsdale in 1948. The program is notable for the fine participation on the part of both profession and general public.

The program of detection and diagnosis in operation in North Carolina was discussed. Here, in examination of 2500 persons, including patients with symptoms as well as apparently well individuals, 109 cases of clinically diagnosed cancer were detected.

At the University of Minnesota, the program of cancer detection places considerable emphasis on the possibilities of detecting increasing numbers of intestinal-tract cancer. The examinations, which are performed on persons more than 45 years of age without symptoms, include the usual detection procedures, and over and above those, and examination of feces for occult blood, a proctoscopic examination, and a histamine test for achlorhydria. Patients who have a positive occult-blood reaction or are either hypochlorhydric or achlorhydric are then given a roentgenological gastrointestinal examination. (When a patient applies for an appointment for examination, he is sent instructions which include information showing the necessity of a meat-free diet for three days previous to the examination and also information as to how to prepare himself for the examination.)

Of 1580 persons examined, eighteen cancers (or 1.2 per cent) were detected. Twelve of the eighteen were early cancers without any evidence of metastases. The distribution was: stomach, two; rectum, six; cecum, two; skin, six; esophagus, one; and leukemia, one. Occult blood was noted in the following lesions: cecum, two; rectum, one; and sigmoid, one.

Emphasis was placed on the value of finding occult blood in the stool as an indication of gastrointestinal cancer. Sugarbaker stated that, in their clinic, they had discontinued routine gastrointestinal roentgenological studies and use a stool examination performed on a glove-adherent specimen to determine which patients should have this study. In his experience, 20 per cent of the people who had a positive reaction for occult blood in the stool were found to have cancer of the gastrointestinal tract; nor had he ever seen a cancer of the stomach that did not have a positive occult-blood reaction in the stool.

Branch added that in the experience of the representatives of the American College of Surgeons attending regional meetings, 80 to 85 per cent of the cases of cancer of the stomach discussed had had a positive reaction for occult blood in the stools.

Cytological examination as a method of case finding in cancer of the stomach was reviewed. It appeared that new techniques in concentration, not yet publicized, may offer possibilities of improving considerably the results of cytological examinations for this particular site.

The discussion went on to the detection of early cancer of the lung. Mass chest roentgenological surveys seemed to offer the best method of detecting early cancer of the lung. It was recalled that, in the Panel on Cancer of the Lung, it was reported that the survey in Minneapolis is expected to yield one case of primary cancer of the lung in every ten thousand people examined.

No figures are available on the results of the mass chest roentgenograms carried on by the various branches of the armed forces during the war. However, the Navy has tabulated its findings since the war. Among 819,416 service personnel examined, twenty-one tumors of all types were detected in the lung, excluding three pulmonary cysts. Sixteen tumors, again excluding three pulmonary

cysts, were detected in 185,826 civilian personnel. It was pointed out that the civilian personnel represents an older age group and naturally would be expected to yield a higher number of cancers per thousand. In advocating these mass surveys, Overholt reported that, at the present time, he is seeing approximately one intrathoracic tumor per week that was found by a survey roentgenogram. The vast majority of these are resectable lesions.

If such surveys are to accomplish anything, they must be accompanied by a complete and prompt system of follow-up. The present policy of some roentgenologists to watch uncertain lesions over a long period of time was condemned. The results of a mass chest roentgenological survey conducted by the Illinois Department of Health are an example of this. Of 162,731 roentgenograms taken, 332 were suspicious of neoplasm. Six of the suspicious cases were not available because of location. Contact was lost with an additional seventy patients because the physicians in charge failed to respond. Finally, the information on some of the replies received was so meager that they were worthless. Hence, of the original 332, adequate information was obtained on only 193 cases. Indeed, by the time the follow-up was completed six of the eleven patients with proved malignant tumors were dead.

It was pointed out that the best yield of cancers of the lung per individual examined could be obtained by restricting the examination to men more than 45 years old. It was not felt that this should be recommended, however.

The question of serum reactions as screening device revealed that at the present time, there are several such tests available, but, as yet, they have not been able to develop beyond a 75 per cent accuracy. One of the Panel stated that a detection center with which he is affiliated is now using one such test, with the just-mentioned results. The members of the staff of this particular clinic felt that they might be able to improve the accuracy of this test and thus make it more valuable. He pointed out that this test is done in addition to the usual detection center procedures. He felt that it might be worth while to have some other centers take one of the other procedures and make some effort to improve the accuracy of it.

A complete blood count as a case finder in cancer was thought to be of little value in detecting cancers, particularly cancer that might lend itself to cure by virtue of early detection. In some 25,000 blood counts performed in the centers studied by the American Cancer Society, only one case of leukemia was detected. This patient was bedridden within two weeks after the case was found. However, many of the Panel felt they might continue to carry out complete blood counts for general health purposes.

There was some discussion of programs of examination set up to detect several diseases in the course of the one examination.

It was pointed out that when one is considering what procedures should be included in the examination and possibilities of screening patients with regard to age, sex, and symptoms, one should have a clear idea as to the purposes of that particular detection program, because such purposes could have a very great bearing upon the decisions made. The difficulty of comparing results of programs set up with different aims was pointed out. The method of screening in a center differs from any other according to the philosophy of that center, thus resulting in different yields of pathology.

Radium Institutes

The Panel recognized the valuable service given to the cancer patient by the various radium institutes situated throughout the country. Branch pointed out that since these institutes are not included in the program of examination for approval as carried on by the American College of Surgeons, the College does not concern itself with the inspection of these facilities.

Mobile Units

The value of mobile units particularly as a means of supplying specialized medical attention to thinly populated rural areas was attested. The programs in two states with particularly active units were discussed in detail.

The mobile unit in Kentucky, donated by a women's club, might be considered a rolling laboratory, since it has facilities to do complete laboratory studies.

Personnel of the unit consists of a full-time director, a driver, and a nurse. This staff is supplemented by volunteers from the various specialties. The unit will visit an area on an invitation of the local medical society. Service is then provided to any patient having symptoms referable to cancer and bearing a note from his family physician. About 75 to 80 per cent of these patients are indigent. The unit usually spends approximately three days in a particular area and examines about twenty patients a day.

Examination consists of a complete history and physical examination as well as any laboratory procedures indicated. The patient is referred back to his physician who is sent a complete report. Last year this unit saw 1400 people, approximately 40 per cent of whom were found to have cancer.

The Kentucky people indicated that one of the problems in connection with their mobile unit was that of obtaining the services of competent radiologists. Sugarbaker stated that in Missouri, where the organization of a mobile unit is presently being considered, it has been felt that the omission of complicated gastrointestinal examinations from the routine of the unit might speed up operations considerably. The substitution of a simple test for occult blood in the stool with referral of all positives for roentgenological examination would probably detect most of the silent gastrointestinal cancers and the less encumbered unit could cover a great deal more ground.

The Oklahoma unit consists of a converted bus with sufficient equipment to set up four examining rooms. This unit confines itself to attempts to detect cancer. The staff consists of three volunteer specialists and a nurse. This unit also visits a particular area on the invitation of the local medical society. Prior to its visit, the event is publicized locally. Individuals are registered by volunteers and detection examination is performed by the staff. The examination is available to anyone who wishes to attend, regardless of the presence or absence of symptoms. Up to December, 1948, 1107 clinical cases were detected among 6100 patients examined. Of these, 40 to 50 per cent were cancer of the skin. This ratio is approximately that which exists in the cancer clinic in Oklahoma City.

The Florida representatives indicated that they are planning a mobile detection center for rural areas of the state. This will be staffed by a full-time physician, nurse, and secretary.

State Programs

It was the consensus of opinion that a good state cancer control program should have as its basis early diagnosis. This can be accomplished by:

1. Getting the patient to the physician early (Public Education).
2. Getting the physician to recognize the lesion early (Professional Education).
3. Providing adequate facilities for diagnosis.

The availability of tissue-diagnosis facilities was discussed and recognized as a valuable item in the cancer control program. Many states have formal state-wide programs. In general, these state-wide programs can be divided in two groups:

1. Those in which the interpretation is carried out in a central laboratory whether operated by or supported by the State Health Department.
2. Those in which the interpretation is allotted to the pathologists of the state on a fee basis.

This latter type of program is supported by one or several of the following agencies in each state: the State Health Department, the American Cancer Society, the American College of Clinical Pathologists. (In California, there is a formal program in operation by which the pathologists perform the service gratis for indigents. The State Society of Clinical Pathologists announced to the profession and the public that its members would waive their fee in all instances in which the physician waived his fee.)

Many states offer diagnostic service in state-sponsored cancer clinics and in state-university laboratories. The absence of a formal program in a state should not necessarily be interpreted as an absence of the facilities, for in many areas, informal arrangements between the pathologists and physicians adequately meet this need.

It was emphasized that a biopsy is no better than the man who takes it and those men who are active in the operation of such programs were asked to take whatever measures necessary to insure a high degree of proficiency on the part of the physicians taking the biopsies.

The question of the availability of cytological diagnosis was raised. It was pointed out that some series of smears made to detect vaginal flora had yielded from three to five carcinomas in situ in each 1000 patients examined. It was further indicated that this procedure should not be substituted for any of the other known diagnostic procedures, particularly biopsy, and should be considered as an adjunct rather than a substitute for the already established procedures. Several states are planning to set up programs whereby cytological diagnostic procedures would be available. The importance of having the interpretation made by a competent cytologist was stressed. The exfoliative cytology fellowships of the American Cancer Society were discussed. This program, at the present time, supports twenty-three fellowships in exfoliative cytology. These fellowships are for a period of four months and are available to physicians who have completed two years of postgraduate training in pathology. The conditions regarding the length of time necessary for adequate training, as well as the type of men to be trained, are in keeping with recommendations made to the American Cancer Society by the Cytological Conference, which was held in Boston in April, 1948.

The importance of including dentists in a cancer control program was emphasized. Several states now have dentists in regular attendance at sessions of the cancer clinics. At least one state is supporting postgraduate refresher courses on cancer for dentists. Many states now have dentists on their cancer commissions.

The treatment of indigent patients was discussed in some detail. It was quite evident that each state had some arrangement peculiar to its own area. In the majority of areas, facilities (not necessarily always adequate) had been made for the treatment of the state-recognized indigent patient. However, in the majority of areas, the so-called medically indigent patient, who could not qualify for state aid and was unable to meet the cost of care himself, pre-

sented a much more serious problem. In general, this latter group had had to depend upon:

1. Philanthropy.
2. Funds from the American Cancer Society in certain states.
3. The good will of the medical profession and hospitals.
4. Sufficiently lax indigency laws to allow some patients to qualify for treatment, in a few states.

Some of the points brought out in the discussion included

1. Last year, Florida spent $127,386.00 in the treatment of 776 patients.
2. Virginia finances three-days' hospital care for indigents for the purpose of diagnosis.
3. In West Virginia, a patient is the responsibility of the Health Department until the condition becomes terminal, at which time he is turned over to the Welfare Department.
4. A report was given of the activities of the Missouri State Cancer Hospital at Columbia, Missouri which, in the past ten years, has treated 12,000 patients.

A unique situation, where the responsibility within a particular community rests in two different State Health Departments, was discussed.

The problem of terminal care was taken up. Some of the Panel members felt that changing the designation, "terminal care" to that of "advanced cancer care" might be desirable. It was pointed out that this would have a more hopeful interpretation. In general, it was felt that terminal care should take into consideration:

1. The patient and his wishes.
2. The patient's family and their wishes.
3. Society and its wishes.

In some areas, institutions are available for this purpose. In others, the nursing service is provided at home. These facilities are usually supported by the Public Health Department or the American Cancer Society. Representatives from Illinois indicated that that

state is considering converting some of the old county farms into chronic disease homes.

The Montefiore plan for home care, whereby a hospital staff, taking advantage of the various facilities that are available in a large community, is able to care for chronically ill and terminal patients adequately in their homes at a considerably reduced cost and under circumstances very pleasing to the patient, was discussed. It was pointed out that, while there was no lowering, whatsoever, of the standards or amount of service available to the patient, the average cost per day (which is approximately $12.00 in the experience of the hospitals in the area) was reduced to about $3.00 per day. New York City has already instituted such a plan in several of the city hospitals, and several other communities are considering the institution of such services.

PANEL ON CANCER REGISTRATION

Moderators, HAROLD F. DORN, PH.D.
National Cancer Institute, Bethesda, Maryland
E. CUYLER HAMMOND, SC.D.
American Cancer Society, New York, New York
Recorder, DANIEL HORN, PH.D.

The aim of the Panel was defined as the frank discussion of the problems of cancer registration. Is cancer registration worth while? What have been the experiences of those working with existing registers? What plans and recommendations may be charted for the future?

SCOPE

Representatives from various state health departments described the procedures now in use in their own registers. Most of

these have been established only within the past year or two, although in New York and Connecticut they were started some ten years ago. In some states, reporting is "compulsory"; in others, voluntary. In some states, only hospitalized patients are reported; in others, private physicians report on cases not hospitalized by them; and in still others, the register is primarily based on pathologists' reports together with tissue specimens. In some states, the register operates on a state-wide, all-inclusive basis; in others, it is limited to those treated at selected hospitals or tumor clinics, sometimes only at state-subsidized clinics; in others again, it is limited to trial areas consisting of a few selected counties.

Objectives

With such wide variations in approach evident, the Panel turned its attention to the aims of cancer registration. The opinion was expressed that a cancer registration system could not be justified solely in terms of providing a set of figures on cancer morbidity. Sampling studies conducted periodically could probably accomplish this more efficiently and less expensively. It was suggested that the cancer register offers a mechanism for providing special services to patients and to physicians. Through the local register, home care may be provided, if indicated by the physician, and follow-up assistance may be given to the physician. Beyond this is the matter of cancer care. Cancer reporting offers an approach to evaluating what we are doing, assists in the administration of a cancer program, and provides data for research requiring careful records and careful follow-up to learn the life experience of cancer patients. The service, the administrative, and the evaluative or the research aspects cannot be clearly separated.

The problem of follow-up of cancer patients was selected as a particularly crucial aspect of cancer registration. The recommendations of the Panel on the reporting of end results, for example, stressed the importance of complete follow-up in the evaluation of cancer therapy. It was felt that follow-up is part of the responsibility of the physician but that, since he lacks the facilities for doing much more than encouraging the patient to return periodically, the cancer register on a local level can provide him

with this service. Doubt was expressed as to the benefit to the patient of the follow-up in terms of "cure," but the value of continued follow-up in terms of services to the patient was clear.

Since the follow-up is crucial to so many aims of the cancer register, discussion centered around methods for accomplishing successful follow-up. In general, follow-up is conducted through the hospital or through the local health department. Both approaches make use of all sources of information, beginning, of course, with the family physician, and including the use of visiting nurses and welfare agencies. The suggestion was made that the national associations of life insurance companies might be able to assist in providing some information on cases otherwise untraced. The resolution was made that a committee be appointed to request the states to assemble information on the procedures used in obtaining follow-up and to distribute this information to interested health departments. Dr. R. H. Hutcheson, as Chairman of the Association of State and Territorial Health Officers, offered to bring this resolution before that organization for action.

Records

The problem of deficiencies and inadequacies in record keeping was discussed. It was agreed that one benefit of cancer registration as it has been carried on in various states has been a marked improvement in the keeping of records in hospital and tumor clinics. The improvement of the general level of competence of record librarians and clinic secretaries was considered within the scope of activity of the health department. In some instances, the knowledge that someone else was studying and analyzing cancer records has led to a general improvement in the level of medical care available.

Legal Aspects

The question of the legal liability of the physician to a charge of divulging privileged information when reporting cases was raised. In the absence of a law permitting cancer registration, this is properly a question for interpretation by the Attorney-General of each State. In most states, the legal opinion has been stated that

the registration of cancer cases comes within the jurisdiction of public-health-department operations. However, it was pointed out that the advantage of having a law on cancer registration—and possibly the only advantage—is that this question is then settled.

Conclusions

The members of the Panel agreed to the following as representing the judgment of those present:

Cancer registration is a proper function of a state health department, if it is conducted in such a way as to provide a useful service. To provide such a useful service, the primary register is local—whether in a hospital or in a local health department—maintained at the point of service to the physician and the patient, and the state register acts as a clearing house for the service centers.

PANEL ON ADMINISTRATION OF GRANTS IN CANCER

Moderator, John H. Teeter
American Cancer Society, New York, New York
Recorder, J. Edward Spike, Jr.

Brief Outline of the Cancer Program in the United States

The purpose of the panel discussion as set forth in the introduction concerns primarily how the various cancer agencies process their funds. This information should be useful to the divisions and to institutions, since it defines the scope of operation and method of approach for financial assistance.

National Cancer Institute Program

The National Cancer Institute founded in 1937, is unique in being the first of the categorical institutes in the United States Public Health Service. Congress appropriated money for a build-

ing and a modest program of fundamental research. Laboratories were built at Bethesda and a bimonthly journal, the Journal of the National Cancer Institute, was established. The National Cancer Institute program was expanded in 1946 by increased funds for research grants-in-aid and fellowships. For this fiscal year, funds for National Cancer Institute activities have increased to an annual rate of $22,000,000.

The operations of the National Cancer Institute are divided into three branches; intramural research, cancer control, and grants-in-aid and fellowships. The intramural research, conducted primarily in the Institute building in Bethesda is in the fields of biochemistry, pathology, biology, radiology, endocrinology, chemotherapy, and biophysics.

The cancer-control branch of the Institute includes several sections. The state-aid section this year distributed $2,500,000 according to a formula that is based on the total population of the state, the density of the population, the extent of the problem, and the per capita income. The statistics section of this branch conducted cancer-morbidity surveys in ten metropolitan areas of the United States ten years ago and recently reviewed these same areas and is currently analyzing the results. The other sections of this branch are concerned with lay and professional education, which includes the trainee program currently granting traineeships to seventy-two young doctors, in addition to lending technical assistance to state public-health agencies, and conducting field demonstrations. This branch is also supporting industrial environmental-cancer surveys and public-health–nursing activities.

The third branch of the National Cancer Institute, namely research grants and fellowships, is currently administering more than 300 grants-in-aid, approximating $3,300,000 and supporting, in addition, 140 fellowships of both pre- and postdoctoral grades at a cost of about $500,000. The grants for construction of research facilities are handled by this branch.

All grant-in-aid requests made to the National Cancer Institute must be approved by the National Advisory Cancer Council, a nongovernmental group of advisers, which recommends expenditures to the Surgeon General who may approve these expenditures

but cannot overrule a negative action by the Council. Construction grants, which are a matter for Council action, are used to increase laboratory space, thereby accelerating the research program. Congress appropriated $2,303,000 in 1948 and, in 1949, increased this by $8,000,000 of contract authorization, which allows a building to be planned and paid for out of subsequent appropriations supplied by the Congress. Several million dollars of additional construction applications are on hand, for which funds have not been appropriated. The great problem in the field of construction grants is to supply needed facilities but to avoid excess expenditures. The question arises "what is the ultimate research need in the United States?"

Liaison between the National Cancer Institute, the American Cancer Society (the Committee on Growth), and the many other agencies in the fields of cancer research are currently carried out through the office of the Director of the National Cancer Institute.

Because of the new construction program at Bethesda, pilot research is now under way to help establish the future program of the U. S. Public Health Service. This work is being conducted in institutions throughout the country.

Atomic Energy Commission

The cancer-research program of the Atomic Energy Commission is primarily for the determination of potential usefulness of radioactive isotopes in medicine. Several different programs are being conducted concurrently. About one quarter of the money spent by the National Research Council for the Committee on Atomic Casualties is for cancer research. This totals about $323,000 for cancer. About $1,000,000 is being spent in studying radiation as it relates to cancer. An additional $200,000 supplies such radioactive elements as iodine, phosphorus, and sodium, to cancer research investigators in non-Government institutions.

Because of the short life of radioactive compounds, clinical cancer projects and facilities costing $4,000,000 are being established near atomic piles. In addition, the training program and a grant-in-aid program costing more than $600,000 is in operation.

The contracts made by the Atomic Energy Commission originate either in its own offices or come through the Office of Naval Research. Although the latter sponsors no direct cancer grants, many of its research projects are in the field of American Cancer Society grants-in-aid as recommended by the Committee on Growth. Because the Atomic Energy Commission budget for the coming year is approximately $900,000,000, even a small fraction of its work in the field of cancer research will constitute a large contribution to cancer. In addition, the Office of Naval Research, which has as its primary function the support of fundamental research in the basic sciences, supports work that relates indirectly, but is nonetheless important, to the cancer-research program. At least $1,000,000 of the Office of Naval Research annual budget is used for the support of basic investigations in biological sciences that are of interest to the cancer study group.

Damon Runyon Memorial Fund and Babe Ruth Cancer Fund

The Damon Runyon Fund, started by Walter Winchell in the middle of 1947, has collected the large sum of more than $2,000,000 to date. Thirty-five annual grants, totaling $1,700,000, have already been distributed to institutions in thirty states. Applications for money from this Fund should be sent to fund headquarters at 235 East 45th Street, New York City, on forms supplied either by that office or obtained through the Research Service Unit of the American Cancer Society. Damon Runyon money distributed by the officers of the Fund (who are Mr. Winchell and his associates) may be used for research salaries, equipment, and expendable supplies, but not for bricks and mortar. Preference is shown for projects in areas where the funds have been collected.

The Society has received $150,000 for Damon Runyon clinical research fellowships. Application for these may be made on forms supplied by the American Cancer Society. The Committee on Growth will act as adviser to the Society on the selection of these fellows.

The Babe Ruth Cancer Fund, part of the Babe Ruth Foundation, may bring an amount of around $100,000 to the Society,

which, it appears, will be spent for cancer research bed activities. The size of these funds may be increased if baseball contributes the receipts from a day's activity during the coming season.

Jane Coffin Childs Memorial Fund for Medical Research

By a gift in trust of approximately $3,500,000, this memorial fund to be used for cancer research as long as need exists was established in 1937 with headquarters in the Yale University School of Medicine at 333 Cedar Street, New Haven, Connecticut. Funds go for cancer research all over the world. About $200,000 is available annually for a research program, which may be used for fellowships, both basic and clinical, for research conferences and the support of publications, and also for grants-in-aid, which constitute the major part of its program. Applications to the Fund are reviewed by a board of scientific advisers who make recommendations to the board of managers for action.

American Cancer Society

The activities of the Society are supported from the income from the annual drive conducted in April by the sixty Divisions of the Society. Of the money collected, 60 per cent remains in the state in which it was subscribed, and the balance is divided as follows: 25 per cent for the research program and 15 per cent for all other expenses of the Society, including fund-raising and all publicity and educational activities.

From the funds that go to the Medical and Scientific Department within the Society, forty clinical fellowships are awarded at an annual cost of $150,000, in addition to a number of fellowships in exfoliative cytology. Applications for these fellowships are made by institutions to the Society, the institutions being given the right to select suitable recipients for the fellowships.

Since 25 per cent of the money which is collected is earmarked for the research program, approximately $3,200,000 was available for the research program from last year's drive. This money is spent for fellowships, grants-in-aid, and institutional research grants. Through its contract with the National Research Council, the Society is advised regarding a portfolio of grants-in-aid and

fellowships. To make the selection, the Council has established the Committee on Growth, a group of twenty distinguished scientists, mostly physicians, who call on the assistance of approximately 120 research specialists in the fields of biology, chemistry, physics, chemotherapy, clinical investigations, and fellowships, to evaluate the applications which are made to them. Currently, they are recommending $1,600,000 for grants-in-aid leaving approximately $200,000 for fellowships for the annual period beginning July 1, 1949. The grant-in-aid recommendations will not amount to even half the requests for support of cancer research. The recommendations from the Committee on Growth that are made to the Society are referred to the Research Committee of the Society and then to the Executive Committee, which approves the program. The Research Committee also considers applications that are made to the Society for Institutional Research Grants and approves a certain number of these to the extent of available funds.

A recent analysis of the applications submitted to the Committee on Growth shows that their recommendations do not favor one section of the country over another but, if anything, tend to advise the establishment of grants-in-aid in those places where cancer research is more sparsely located.

The fellowship program of the Society was established to increase the available man power. Already 120 man-years of research fellowships have been supported: seventy-four fellowships totaling $525,700 to date. The second approach to research is the grants-in-aid program, which has already received more than $4,000,000 since its inception in 1945. The third approach to the research attack on cancer, namely Institutional Research Grants, was inaugurated early in 1948. Already some thirty-five grants have been made at a cost of about $3,300,000 for support of research in locations in which laboratory investigation, hospital facilities, and teaching institutions, are exerting their combined influence. Research salaries, equipment, expendable supplies, and cancer research beds are supported with these funds. Application is made to the secretary of the Research Committee at 47 Beaver Street, in accordance with the form supplied and should be avail-

able for consideration by the Research Committee in June prior to the 1st of September of the year in which the funds are needed.

A survey is being made of the distribution of all cancer-research funds in each of these three categories. Reports of the survey, both on the American Cancer Society and all other agencies that cooperate with the Society, are available through the Research Service Unit and are distributed periodically to all the Divisions.

State Programs of Administration of Funds Allocated to Cancer

To supplement the local agencies, the Federal government is currently distributing $2,500,000 to fifty-three subdivisions of the United States for lay and professional education, for collection of statistical data, and for support of state public-health programs. The amount of money available to each state is small; 30 states get a maximum of $30,000 each. The formula used by the U. S. Public Health Service in distributing its funds has been examined recently, and it is found that money distributed in all categories shows a slightly higher ratio of funds-granted to funds-requested in areas where research is slight. Such a distribution tends to develop research potential throughout the United States.

Cancer Research Grants

A special panel of research administrators from five medical centers receiving American Cancer Society institutional research grants described the methods used in administering cancer research beds. Criteria are established so that research money is not used to support indigent and terminal patients. The patient who occupies a cancer research bed should be one whose case is particularly worth investigating because metabolic or other studies ought to be carried on to a degree not normally required by usual hospitalization; again, a research bed might be used for a terminal patient so that complete case history may be obtainable. In general, a committee passes on all cases where use of a research bed has been recommended—the committee being made up of heads of departments, especially selected administrative leaders from the research section of the hospital staff.

The total number of cancer research beds in a large hospital seldom exceeds twenty, and the average is about ten. Less than half of these will be for metabolic research which, of course, is most expensive. The panel of cancer research bed administrators speaking about their individual problems agreed in general that $14.00 to $16.00 a day was a reasonable cost for a cancer bed and that, to make the necessary research tests, it might be necessary to assign four employees to each research bed in addition to using the services of the professional staff to a much greater extent than would be required for a normal cancer patient. Exclusive of professional costs, the cancer research bed may require $1500 to $2000 more annually, because of the special diet, care, and laboratory treatment required.

This special panel considered briefly the question of whether their institutions would be interested in a continuance of institutional research funds distributed in the manner of these first grants and all agreed that such money, which is reasonably unrestricted, except for general research purposes, was highly useful and assisted them especially in administrative fashion. Institutional research-grant money used for projects other than research beds allows the administrators to do pilot research and provide for unanticipated apparatus expense and establish short-term research projects which grant-in-aid funds will not provide. Practically all the administrators mentioned that such research might be supported on grant-in-aid money after its first year, providing a suitable program had been developed for this work.

It was also pointed out that a certain amount of prestige is acquired by an investigator whose project receives support after favorable recommendation either by the Committee on Growth or by the National Advisory Cancer Council. It was likewise pointed out that grant-in-aid projects are not entirely inflexible with regard to subject matter and the budget could be revised if intermediate research results indicated this to be desirable. In general, it was agreed that the operations of grants-in-aid and institutional research grants were mutually compatible.

The special panel discussion ended with praise for those institutions where research committees have been established to con-

sider the relation of entire research program of the institution as an integrated program.

Summation of Panel Discussion

With the government in the research business to an extent involving more than $1,000,000,000, it was pointed out that research was "big business," and that institutions throughout the country should be prepared to carry their respective loads. Contrasting with this large sum in support of research, it is noted that life insurance companies paying out death benefits of $482,000,000 in heart-failure deaths and $241,000,000 in cancer cases in 1947 allocated only $1,225,000 for research, and this was entirely for research in heart conditions.

Possible future cancer research needs were briefly mentioned. The possibility of establishing, through joint action by American Cancer Society, National Cancer Institute, and National Research Council co-operation, a central agency for evaluating cancer cures was mentioned as a solution to the elimination of cancer quackery. Also, it was suggested that terminal cancer patients not be abandoned by the medical profession as hopeless but that all research efforts be continued until death.

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In general, tumors are not indexed under definitive names, e.g., "Chondrosarcoma." Reference should be made to the pertinent site, e.g., "Bone, tumors of, classification of," and then to the text for the particular tumor.

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