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NORMAL AND MALIGNANT CELLS[1]

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THIS evening I propose to discuss the proposition that malignant cells are permanently altered cells. They are new types or species of cells that arise in the body from normal, usually adult, cells which have been altered by environmental influences or agents of one sort or another. The alterations appear to be irreversible in the body and in vitro under the ordinary conditions in which the cells live and multiply. It is conceivable that just as normal cells can be converted into malignant cells by an extraordinary environment, so perhaps malignant cells of one type may be changed into other types (of malignant cells) or reconverted into normal cells by a different environment. The pathologists speak of tumors changing. This may be explained either by an alteration of the malignant cell or by the gradual overgrowth of one special type among two or more types that were originally present. After malignant cells are once established they multiply independently of the special environment or agents which produced them, as is amply illustrated by the growth of metastases, by serial transplantations from animal to animal and by serial in vitro cultures. They can multiply indefinitely *in vivo* and *in vitro*. Many are cytologically different, that is visibly different from normal cells of the type from which they arose.

Speculations on the origin of malignant tumors or neoplasms have extended over a long period of years. Advocates, led astray by their own pet theories, have fought for this and that idea. In my student days there came to Baltimore pleaders for the protozoan origin of cancer, and following this a distinguished protozoologist worked out the life history of the

[1] Presidential address, read before the American Association of Anatomists, at the St. Louis meeting, April 19, 1935.

cancer-producing protozoan. Peculiar bodies seen in some carcinoma cells led to this view, but since they are not present in various other types of cancer the idea soon died a natural death.

Bacteria have in turn been pushed to the front as the causative agents of cancer. Bacteria like protozoa have been eliminated from the race, although there are still a few who believe that they can produce malignant tumors. The work of Irwin Smith on the tumors in plants produced by the *bacterium tumefaciens* gave an added impetus some years ago to the idea, when it was found that remarkable tumors could be produced in animals with a similar organism recovered from a human breast carcinoma. No evidence has appeared that the cells of such growths can multiply independently of the organism and until such evidence is conclusively demonstrated these tumors should not be classified with malignant ones.

There are still investigators who maintain that tumors are probably due to viruses. They state that viruses may produce every type of cell change from pure necrosis to almost pure cell proliferation. They point to a series of virus epithelial tumors ending with non-filterable ones and malignant epithelioma and to a series of connective tissue tumors, beginning with ones produced by filterable viruses and ending with non-filterable fowl and mammalian sarcomas, and ask where a line is to be drawn between infectious tumors and so-called true tumors or the ones that we would say are not dependent on the presence of any infectious agent for their origin and growth. Many attempts have been made to recover viruses from true mammalian tumors and to reproduce the tumors with filtrates but so far without success, although claims to the contrary have frequently been made. The participation of viruses in the production of what we might term true tumors is not easy to eliminate, but a provisional line can be drawn between them. The virus tumors can be produced by cell-free filtrates but not the malignant ones. Some day it may be shown that some of the tumors now considered malignant are in the virus class. Another profound difficulty exists in the uncertainty as to the very nature of viruses. Are they living organisms or merely chemical substances? But even with this uncertainty we can separate the true tumors from the turmoil by depending on whether cell-free material from them will or will not produce the tumors. This leaves the famous Rous chicken sarcoma on the virus side, where it probably belongs.

Although one might explain the multiplication of malignant cells by the presence of a virus which increases with cell increase it is difficult to understand, if a virus is always present, why after tumors are once started no additional cells of the host are converted into malignant ones. In the growth of metastases, from epithelial tumors, in the spleen, the lungs and other organs one can see plainly that they are composed of descendants of cells from the original tumor; they stand out in marked contrast to the surrounding tissues. The same is true when tumors are transplanted from animal to animal. One can back out of the situation by assuming that the virus is so intimately attached to or secured within the cells that it can not get away to neighboring cells while it can readily be transmitted to daughter cells where it multiplies for the next generation of cells. Most tumors have many dying and dead cells offering a chance for the release of the virus, but this does not seem to result in changing neighboring normal cells into malignant ones. Having somewhat arbitrarily ruled out all the virus tumors from our class of malignant ones we will proceed to other tumor-producing agents.

One of the most, if not the most, prolific sources of induced animal sarcomas are the tapeworm cysts of the liver resulting from the feeding of tapeworm eggs of the cat to rats. At the Institute of Cancer Research, New York, over 4,300 such tumors have been produced in this way. The larvae pass from the stomach to the liver and induce cyst formation about them. In the walls of such cysts there frequently develop after many months typical sarcomata of one sort or another. There has been considerable speculation in the minds of people who have had anything to do with tumors as to the causative factors involved. Long-continued irritation, the favorite factor of the clinicians, the possibility of a virus being carried along with the larvae and of chemical products of the latter have all been considered without arriving at a definite answer. From none of these tumors has a filterable virus been obtained that will reproduce the tumor. A number of these tumors have been transplanted from animal to animal for considerable periods of time, and we have had the privilege of studying several. One of them has been carried for over 4 years in vitro. They fall readily into the class of malignant tumors.

The occurrence of certain occupational cancers led two Japanese workers to experiment with tar. They succeeded in producing cancers on the ears of mice after repeated applications over a long period of time. This has been many times repeated and led to testing out of purified products from tar. During the past few years, some English workers have succeeded in producing both carcinoma and sarcoma in mice and rats with such products, namely, 1:2:5:6 dibenzanthracene and related substances.

These chemically pure substances produce typical malignant tumors in a relatively short time, six months or less with the dibenzanthracene and in a considerably shorter time with methylcolanthrene. The sarcomata were produced by injecting subcutaneously under

strictly aseptic precautions very small amounts of dibenzanthracene dissolved in lard. These experiments would seem to eliminate the direct participation of living agents of any sort as the cause of the tumors and also to eliminate the old idea that true tumors are produced by the uncontrolled multiplication of normal cells induced by the presence in, on or near the cells of a living agent. It is interesting to note that these recently discovered tumor-producing substances are chemically related to the oestrous-producing hormones. So now it is quite stylish to work on the hormonal origin of tumors. With the new group of tumors produced by chemically pure substances 1:2:5:6 dibenzanthracene there is a fine opportunity to test out the filtrates. My guess is that such tumors will not give filtrates with either virus or chemical agents, which can reproduce the tumor especially after they have been transferred for a few times from animal to animal.

Having eliminated for the time being at least the idea that malignancy is due to living agents of any sort we must also eliminate the continued presence of some chemical agent that might be responsible for the peculiar properties of malignant cells such as (1) the uncontrolled growth in the body, (2) the transplantability to other animals of the same strain or species, (3) the peculiar cytological characters which serve to distinguish them from normal cells and (4) the maintenance in vivo and in vitro of these characteristics for generation after generation and year after year.

It is conceivable that the continued presence in, on or near normal cells of some special chemical agent might be responsible for the peculiar properties which are assigned to malignant cells. Let us suppose that four fibroblasts are converted into malignant cells at the site of injection of 4 mgm of dibenzanthracene, which is enough to induce a tumor. In a few weeks a tumor 40 mm in diameter will result with something like 4 billion cells. There will then be about one billionth of a milligram per cell to keep it in line. Suppose a number of metastases have developed in the body, since they consist of descendants of the original tumor cells, the allowance of dibenzanthracene per cell may then be not one billionth of a milligram but one two or one three billionth of a milligram. One may assume that that is enough of the agent to keep the malignant cells malignant. We are so accustomed to talking in billions these days that billionths may not seem so small.

It seems improbable, however, that such an agent could remain present in sufficient quantities to continue potent during the many cell divisions involved either in vivo or in vitro when cells are transplanted from animal to animal or culture to culture over a period of years, unless there was a continued addition to the original amount of the chemical agents which started the cells off in their new line at the time of the origin of the tumor. In the course of a month rat sarcomas often attain diameters of 40 mm or more. From such a tumor 100 rats could be inoculated. At the end of another month 10,000 could be inoculated. At the end of a year the astronomical number of 10^{24} rats would be running around, each with 4 billion tumor cells. If the chemical agent were as simple as water there would be only about one half a molecule per cell. So we can dismiss the notion that the presence of some of the original chemical agent keeps the cells malignant, or keeps otherwise normal cells in this condition.

It is possible that the chemical agent could be supplied by the host to keep the cells malignant during their multiplication in the original animal in which the original tumor arose. It is rather improbable that animal after animal for generation after generation could supply the agent to keep the transplanted tumor cells going and next to impossible for such a chemical agent to be introduced into a long series of in vitro cultures automatically with the medium where for example rat tumor cells are cultivated in horse serum, chick embryo juice, chicken plasma and a saline solution.

There is another idea which we might dwell on for a moment, namely, that the original agent might stimulate the cells to produce more of that particular agent and thus maintain the malignant nature of the multiplying cells. This is visionary and would imply an alteration of the cell.

I have omitted the x-ray cancers and the various spontaneous malignant tumors of man and animals. The very designation of the latter as spontaneous indicates that we know nothing definite about their origin. The x-ray cancers presumably arise as a result of injury to the epithelial cells, the presence or supposed presence of a ubiquitous virus is difficult to rule out, but the probabilities are that they too arise from altered cells.

These considerations thus seem to eliminate the idea that malignant cells are merely normal cells under the continuous stimulation of some extraneous agent. The most plausible alternative concept is the one we are considering, namely, that malignant cells are permanently altered cells derived from normal cells.

We have little definite information as to the steps in the alteration. There are as already noted a number of known agents which can change normal into malignant cells. The known agents take weeks or months to produce malignant cells or to produce tumors that are palpable. This may indicate long-continued action and slow gradual change of one or a few cells or it may indicate that many cells are altered in various ways and that only rarely is the alteration of such a char-

acter as to produce a malignant cell. Dr. Mendelsohn, working in our laboratory, has found that fourteen-day tapeworm cysts of the liver show all sorts of abnormal mitotic figures similar to those found in tumors, yet palpable tumors do not appear until 8 to 22 months later. What relation these early abnormal mitoses bear to the origin of tumors is uncertain, but it shows that the cells are already being upset long before tumors appear.

Having succeeded in getting the malignant cell started as an independent type of cell let us consider some of the differences between normal and malignant cells.

One of the outstanding differences between normal and malignant cells is controlled versus uncontrolled multiplication in the body. All normal cells are under control of some sort. The nature of the control mechanism is obscure but fundamental for all multicellular organisms. It begins at the 2-cell stage and continues throughout life. So familiar are we with the manifestation of this law that deviations from it at once attract attention. The control is elastic, it permits of increase and decrease of organs and parts through use and disuse, disease and recovery, injury and healing and regeneration. The end result is a return or an attempt to return the part to the normal condition. The swellings that develop in some infections from induced cell multiplication are sometimes remarkably like malignant tumors, but when the infectious agents are overcome the tissues tend to return to normal.

Malignant cells, on the contrary, continue to multiply like parasites, frequently spread through the body and continue to increase in number until they kill the host. This uncontrolled multiplication of the malignant cells sets them apart from all normal cells. All tumors have this important characteristic of uncontrolled growth in common. Some very malignant ones grow and spread like wild fire and kill in a short time, others grow slowly and benign tumors may last for years and years as though their cells were almost under control. All gradations between these extremes are found. We are concerned more especially with the cells of malignant tumors, rather than with those of benign ones.

Malignant cells tend to grow in a disorderly manner, especially the more malignant ones, and produce structures with little resemblance to normal organized tissues. Their metabolism is altered. The production of normal secretions is debatable. The usual assumption is that they produce harmful rather than useful metabolic products. The increase in malignant cells thus can not be attributed to a demand by the rest of the body for their secretions as with normal ones.

There are, as you know, quite a number of transplantable rat and mouse tumors that have been carried for years by serial inoculations of small pieces of the tumor from animal to animal of the same species. Several tumors have been thus maintained for many years, ten, fifteen, twenty and even thirty years. The essential part of the technique is that living malignant cells shall be inoculated into animals of the same species or strain. There are some tumors that take in nearly 100 per cent. in almost any strain; others take in smaller percentages and still others take in only the particular strain in which they originated. Of the many spontaneous and induced tumors of rats and mice only a small percentage has been found to be transplantable. This is partly because some have not been tested or have been given only a perfunctory trial and partly because it often makes a very great difference as to whether or not inoculations were made into animals of the same strain and on the purity of the strains. Many of the failures may be attributed to the fact that the tumors tested originated in such mixed strains that no two animals had anything like the same genetic constitution. Recently a series of tumors were produced in five inbred strains of mice and one "stock" mixed strain with 1:2:5:6 dibenzanthracene by Andervont. Some mice of each strain developed tumors. There were about 100 per cent. "takes" when tumors were transplanted into animals of the same strain in which the tumor originated and 100 per cent. negative results when transplanted into the other strains. The subsequent serial transmission of these tumors to animals of the same strain were about 100 per cent. successful and 100 per cent. negative into animals of another strain. This illustrates the importance of dealing with pure inbred strains and may explain the negative results in the many attempts of serial transmission in mixed strains where no two animals have the same genetic constitution.

It is well known that in mammals most normal cells and tissues do not live long when transplanted to another animal of the same species. This curious individuality of each animal's tissues has received considerable attention from St. Louis' pathologist, Leo Loeb. The surgeons are well aware of this specificity from the universal failures with skin grafts from one individual to another. Attempts made to so cultivate skin epithelium that it will lose this individual specificity and thus be transplantable to any one have not been successful. The failures of ordinary normal tissue transplants from animal to animal and man to man are probably associated with differences in genetic constitution. No two animals of mixed colonies and certainly no two humans, except identical twins, have the same genetic constitution. When we turn for example to pure inbred strains of guinea pigs the success of cross transplantation of normal tissues increases with genetic identity as shown by Leo Loeb and his asso-

ciates. Brother to brother transplantations in an inbred guinea pig strain were nearly equal to autotransplantations. In inbred strains of rats, however, through brother and sister matings up to the forty-seventh generation, there seemed to be no distinct diminution in the reaction against transplants within the inbred family as compared with non-inbred ones.

On the whole, however, malignant cells are much more readily transplantable from animal to animal than normal ones, especially among animals of mixed genetic strains. This is unfortunate in view of the desirability of supplementing defective organs of one sort or another by the transplanting of normal organs or parts of normal organs into individuals with defective ones. Efforts are being made to train normal cells into transplantable ones. If they could be made slightly malignant or, perhaps better still, converted into benign ones in vitro the problem might be solved. At present we are still much in the dark as to why malignant cells are transplantable and normal cells not.

The behavior of malignant cells when cultivated over long periods of time in vitro is a strong argument for the idea that they are permanently altered cells. The malignant cells of a mouse adenocarcinoma have been carried in pure cultures for seven or eight years by Fischer without losing their malignancy. Carrel and Ebeling carried two rat sarcomas for 16 months with the same result. We have at present pure cultures of malignant cells from six different rat sarcomas that have been cultivated in vitro from one and a half to over 4 years. They have retained their essential cultural and cytological characteristics and their malignancy during the cultivation.

These sarcomas are all well-established transplantable tumors that had been carried on in rats for generation after generation for 3 to 20 years. Our studies on the behavior and cytology of the malignant cells from these tumors in simple hanging drop cultures began after the tumor had become well established in animals and were continued for some time before serial cultures were undertaken and were also carried on parallel with the serial cultures. The many series of cultures in simple hanging drops of chicken plasma, rat plasma and combinations of the two, with and without neutral red, enabled us to become quite familiar with the cultural and cytological characteristics of the malignant cells. Both the outgrowth patterns and the cytological characters of the cells are different from those of normal cells and from one another.

Attempts were made to cultivate a number of other transplantable rat and mouse tumors in serial cultures, only to have the cells die out on our hands after a few generations. I attribute the failures to lack of suitable media and imagine that some day it will be possible to cultivate almost every type of malignant cell indefinitely outside the body.

The six tumors which we have carried on in serial cultures comprise three round cell sarcomas, one spindle cell sarcoma, one rhabdo-myosarcoma and one polymorphous (mixed) cell sarcoma. The small pieces of tumor tissue with which the serial cultures were started contained many macrophages, monocytes, lymphocytes, fibroblasts and endothelial cells in addition to the malignant cells. Thus the primary cultures were mixed and sometimes contained all the above types of cells in the migratory zone. After a varying number of transfers we found without any special effort that the colonies contained only malignant cells with the possible exception of those from the polymorphous cell sarcoma. Various combinations of chicken plasma, dog plasma, human plasma, rat serum, human placental serum, horse serum, beef embryo juice, chick embryo juice and saline were used.

The colonies were carried in two types of cultures, the large sitting drops by Mrs. Gey and the roller tubes. In the former the cell colonies were replanted every four days into fresh clots without a supernatant nutrient fluid. The colonies in the roller test-tubes were replanted at varying intervals of 4 to 21 days, depending upon the condition of the colonies. The roller tube cultures have a thin clot of blood plasma lining the tube in which the colonies grow and a supernatant nutritive fluid which was changed every 4 days. From time to time simple hanging drop cultures were made from the large colonies for cytological examination. Rats were also inoculated every few months with one to several colonies to determine the malignancy. Up to the present the cells have maintained their essential cytological characters and malignancy. Some are not in as good condition as those taken directly from tumors, while others seem to show no ill effects from their prolonged life in vitro.

The inoculations into rats of the pure colonies of malignant cells have resulted in about the usual number of "takes" giving rise to typical tumors. Cultures from these tumors displayed the typical array of malignant cells, macrophages, monocytes, lymphocytes, fibroblasts, etc. The malignant cells were similar to those from tumors that have been carried on from animal to animal during the same period and the period preceding the serial culture experiments. The malignant cells, not in good condition, frequently produced typical tumors, and cultures from them revealed good healthy malignant cells, indicating their revival after inoculation into animals, and also that they were not seriously injured or modified.

In a few instances serial cultures were started from tumors produced by the inoculation of pure cultures.

These serial cultures gave in due course of time pure strains of malignant cells which in turn produced typical tumors. The tumors produced by the pure strains of cells are transplantable as were the progenitors of the tumors of the pure strains and some have been carried on from animal to animal for a number of generations.

The conclusive evidence that one has obtained the malignant cells is their recognition in cultures, their cultivation in pure strains, the production, on inoculation of the latter into animals of the same species or strain, of typical transplantable tumors and the recognition again of the malignant cells in cultures of such tumors. These conditions have been fulfilled with the above six rat sarcomas. The fact that the malignant cells have maintained, through all the vicissitudes of prolonged serial cultivation, their essential and recognizable cytological as well as their cultural characteristics which are peculiar for the malignant cells of each tumor and are different from normal cells speaks strongly for the idea that they are permanently altered cells that bred true.

Let us now turn to the most difficult task of all, namely, to give you in words an adequate idea of the cytological differences between normal and malignant cells and between the various malignant cells themselves. Our observations are based on the study in tissue cultures of the malignant cells of 27 rat and mouse tumors. Some have been studied again and again over a period of years, others in only a few or a single set of cultures.

Let us consider sarcoma cells with which I am most familiar. Supposedly they come from fibroblasts or other cells derived from the middle germ layer, and until we know better I shall compare some of the malignant sarcoma cells with fibroblasts. If I could lay before you a series of photographs which I have in Baltimore of living fibroblasts and of living sarcoma cells at a magnification of 1,000 diameters you would see at once that malignant cells are different from fibroblasts, and then you would begin to see that the sarcoma cells from each tumor were different from those of every other tumor. If I could put before you groups of photographs of a number of cells from each of the tumors you would see that the cells in each group varied considerably, yet those from any one tumor are enough alike and enough different from those of any other tumor to be assigned to their own kind. Fibroblasts would also show similar variations. The cells from any one tumor vary like the leaves on a tree, no two are exactly alike, yet a red oak leaf is a red oak leaf, and a maple leaf a maple leaf, in spite of considerable variation in size, shape, etc. You would also note that the cells from the spindle cell sarcomas are more like each other than they are like those from the round cell sarcomas or the polymorphous and mixed cell sarcomas. It is probable that the malignant cells of spindle cell sarcomas come from fibroblasts. It is not so evident from what cells some of the other sarcomas arise.

The general architecture of malignant fibroblasts is similar to that of normal ones and is best seen in cells flattened out on the coverglass. The spread-out normal resting fibroblast has a very delicate transparent cytoplasm. It is often difficult to detect the edge in some places, rather easy in others. The more or less excentric oval nucleus is also clear and transparent; the small nucleoli are the only things to be seen in it. The nuclear membrane is extremely delicate. At the center of the cell is the centriole, not usually detectible in the living cell and rarely seen in photographs. About this center are a few granules and mitochondria, then a little further out are the fat globules and more mitochondria. Scattered granules, fat globules and mitochondria occur in the peripheral ectoplasm and cell processes. The mitochondrial threads tend to be radially arranged about the centriole. This is about the picture one gets of normal living fibroblasts that are spread out on the cover-glass in fresh cultures during the first 24 hours. The whole arrangement becomes emphasized in older cultures. The finely granular central area surrounding the centriole becomes greatly enlarged. About this is a zone of neutral red stainable granules and small vacuoles; about this in turn is usually a heavy zone of fat globules and about the periphery of the cell is the relatively clear ectoplasm. The mitochondria are not confined to any one zone but become more or less radially arranged in reference to the central area and centriole. The nucleus is pushed to one side against the fat globule zone by the enlarged central area.

Malignant sarcoma cells have the same general arrangement. The cells from some sarcomas even in the 24-hour cultures have large central areas, some have heavy fat zones, others very little fat, some have scarcely any neutral red granular zone, others have quite a good one, but it is rarely as prominent as in normal cells. In general the resting malignant fibroblasts as seen in cultures have less transparent and more granular cytoplasm, larger nuclei in proportion to cell size, heavier nuclear membranes and larger and more irregular nucleoli than normal fibroblasts. The arrangement of the fat globules is often a little different. The malignant cell accumulates as a rule fewer and smaller neutral red granules than normal cells. The mitochondria tend to be smaller and more numerous than in normal ones. The nuclei of malignant cells are much more frequently deeply indented on the central side than those of normal ones. This pocket may be quite extensive. A superficial examination of

such a nucleus might lead one to think that the nucleus was highly granular, as the nucleus forms a thick shell like a pushed-in rubber ball, around the pocket filled with granular cytoplasm of the central area. The nucleolar material is sometimes so extensive and broken up into granules that it gives the nucleus a granular appearance, but one can still see plenty of clear nucleoplasm.

Malignant cells are usually larger than normal ones but not always. Owing to the variations in the amount of spreading out on the coverglass about the only safe way to compare cells is to measure ones that have become spherical. The malignant cells of some tumors are fairly uniform in size, while those from others vary enormously, such variations being characteristic for the tumor. There is something very difficult to define but rather characteristic about the manner in which normal and malignant cells spread out on the coverglass that enables one to distinguish at a glance a normal from a malignant cell and the various malignant cells from one another. Malignant cells often have peculiar ruffle pseudopodia somewhat like those on macrophages but different from the wavy edge of normal fibroblasts. They are the organs for pinocytosis, a common habit of malignant fibroblasts. Pinocytosis is a fancy name for cell drinking, a habit normal fibroblasts rarely indulge in. The macrophages, as I pointed out long ago, are great drinkers. Phagocytosis is also more common with malignant fibroblasts than with normal ones. One could go on for some time relating how this and that type of malignant cell differs from a normal one, much to your confusion.

There is no one startling character which serves to distinguish malignant cells from normal ones, yet among those which I have studied in tissue cultures, and they comprise ones from 27 different tumors from the rats and mice, there is no great difficulty in recognizing most of them. The whole cell is more or less altered. It is a sort of a constitutional thing. There are all sorts and combinations of slight differences between normal and malignant cells and between the various types of the latter. Long familiarity, as with many other things in life, enables one to distinguish at a glance differences and qualities that a long and arduous description would fail to reveal.

There are, however, some malignant cells such as those from the spontaneous adenocarcinomas of the mouse that seem to have no visible malignant characteristics, according to Mrs. Lewis and Strong.

It will be noted that the term mutant has not been mentioned for these altered cells. A mutation, according to the geneticists, depends on some alteration in the genes or chromosomal complex. In common parlance mutant may be used for any sort of alteration that descends from generation to generation, but the general inference has been when speaking of malignant cells as mutants, to assume that their peculiarities are due to gene alterations. Up to the present there is no proof that chromosomal or gene alterations are responsible for the various malignant cells and it would be very difficult to prove that the genes were altered even if they were. The chromosomes are numerous and small, and it will be a long long time before one can see in them anything at all comparable to what the giant chromosomes of the salivary gland of the fruit-fly reveal.

Malignant cells are notoriously afflicted with chromosome troubles. The amazing variations in the chromosome picture of fixed and stained preparations in sections and cultures hold the eye to the exclusion of the rest of the cell, which does not show much of anything anyway in such preparations. It is often worse than looking for the soft parts in a fossil.

More mature consideration of such facts as we have at hand has convinced me, temporarily at least, that the chromosome abnormalities are only the manifestation of a more subtle trouble of the cell. A boil, a fever and leucocytosis are manifestations of an infection, not the cause of it. A short survey of the chromosomal abnormalities of malignant cells may convince you also that they are secondary phenomena to other changes in the cell rather than primary, even though one has not determined exactly the primary change.

Most if not all tumors show cells with abnormal chromosomal complexes and mitotic figures as well as normal ones. Some tumors display an astonishing array of abnormal mitotic figures that are repeated in a long line of serial transplanted tumors. There occur cells with the haploid, diploid, tetraploid and greater numbers of chromosomes. In addition cells are encountered with a few more or less than the above numbers. In these tumors all sorts of irregularities of cell division abound, such as division into three or even four equal or unequal cells with an equal or unequal number of chromosomes. Cells with such variable numbers of chromosomes frequently undergo mitoses and are encountered in cultures, especially those from the Walker spindle-cell sarcoma 338 made from time to time over a period of years. This leads one to suspect that the exact full complement of chromosomes or any exact multiple thereof is not essential to the continued life of a malignant cell. One wonders if after differentiation is completed the exact number of chromosomes is essential for the continued life of any somatic cell. In cultures of embryonic chick tissue one occasionally encounters normal cells with abnormal division figures such as tripolars where the chromosomes have every chance of being unevenly distributed to the three daughter cells. I have been told that

abnormal mitosis are sometimes encountered in inflammatory areas.

The unequal distribution of chromosomes during tripolar divisions of malignant sarcoma cells comes about from the usual condition of unequal size of the three limbs of the Y-shaped metaphase plate and also from the fact that total numbers of chromosomes in the three-limbed metaphase plate is usually not sufficient to give each of the three daughter nuclei the normal number of chromosomes. The interesting thing in connection with the variable number of chromosomes in such cells is that the tumor cells from any one tumor are all essentially alike in spite of the fact that some of them are small and have small nuclei and few chromosomes, that some are intermediate and have intermediate sized nuclei and that some are large, have large nuclei (giant nuclei) and more than the normal number of chromosomes. Since otherwise the general cytological and cultural characteristics of such malignant cells of any one tumor are similar to one another and to those with the normal number of chromosomes it would follow that chromosomal variation has no particular effect on the cytoplasm except increasing or decreasing its volume.

Malignant cells with two to several nuclei are frequently encountered in cultures from several of the tumors without noticeable change in the character of the cytoplasm. The nuclei in such cells usually vary in size and often no two nuclei even in the same cell are of the same size. From studies on the tripolar divisions with unequal distribution of chromosomes and cytoplasm to daughter cells we find that the nuclei resulting therefrom vary in size according to the number of chromosomes. It seems probable, therefore, that multinucleated cells having two or more nuclei of unequal size have not two, three or four times the number of chromosomes but variable numbers something more or less than 2, 3 or 4 times the normal number yet the cytoplasmic characteristics remain unchanged. The same thing applies to various sorts of normal cells with two or more nuclei.

Abnormal distribution of chromosomes may also come about through the occurrence of lagging and aberrant chromosomes. Not infrequently one or more chromosomes fail to get into the metaphase plate and when the two groups of chromosomes pass to the daughter nuclei they are not included, but are left in the cytoplasm where they form small chromosomal vesicles. A somewhat similar displacement of chromosomes occurs when one or more chromosomes fail to pass to the daughter nuclei from the metaphase plate. Such lagging chromosomes are left behind, outside the daughter nuclei where they also form small vesicles. The ultimate fate of such chromosome vesicles and cells containing them is unknown, but the latter retain their essential cytological characteristics as long as they have been followed. The cytological characteristics of malignant cells after they have become differentiated are thus apparently not dependent on the maintenance of the exact complement of chromosomes. From this it seems probable that chromosome or gene alterations have nothing to do with the origin of malignancy. The variable distribution of chromosomes in malignant cells is probably secondary to alterations of other parts of the cell, the cytoplasm and centrosomal system.

That chromosome troubles are not primarily due to gene alteration is also borne out by the experiments in our laboratory, on normal cells in cultures, of Mrs. Lewis on the effects of fluorescent X, of Rosenfeld on the effects of ether and ammonia and of Whitman on those of radium.

Fluorescent X causes the terminal ends of some of the chromosomes to adhere so that they fail to completely separate at the usual time during anaphase. This results in lagging chromosomes and unequal distribution to the daughter nuclei. In some cells one or more lagging chromosomes were omitted from each daughter nucleus, in others the two sister chromosomes passed into one daughter nucleus. In strong concentrations of the dye the chromosomes failed to separate into two groups. Cytoplasmic division ensued, however, and the chromosome complex was mechanically squeezed into two masses to form the nuclei for two daughter cells with an unequal distribution of chromosomes.

Rosenfeld found that when normal cells in metaphase or anaphase were subjected to ammonia the progress of mitosis was interrupted. Sometimes the chromosomes became aggregated into a single nucleus with the tetraploid number and on the return to the normal culture medium formed a large resting nucleus normal in appearance in a large cell in which the cytoplasmic division was suppressed. Sometimes after the initial aggregation the chromosomes became scattered in the spindle area and after the return to the normal medium, when cleavage occurred the daughter cells contained unequal numbers of chromosomes. He also found that ether produced abnormal mitoses, following an initial aggregation of the chromosomes. On return to normal medium a variety of events occurred. Sometimes cleavage was suppressed, as with ammonia, and a large nucleus with the tetraploid number of chromosomes or a binucleated cell resulted. Radium also produces abnormal mitoses, lagging and aberrant chromosomes and unequal distribution to the daughter cells, yet radium has never been known to produce tumors in man in spite of extensive use.

I do not care to push too hard at the idea that malignancy is due primarily to cytoplasmic alteration

rather than chromosomal or gene ones, but I am inclined to consider it from that angle at present. The important point which I wish to emphasize is that malignant cells are permanently altered cells that breed true. They are new types or species of cells. This undoubtedly holds for the malignant cells of spontaneous as well as induced tumors. Many spontaneous tumors seem to arise *de novo* as the result of unknown factors at play within the organism entirely unconnected with any outside environmental effects. Others seem to arise from a combination of environmental and autofactors as in locations of chronic irritations. I often wonder if irritable people are more subject to brain tumors than placid ones. If autofactors can produce malignant cells perhaps they can also produce useful alterations. Who knows but that something of this sort has played an important rôle in our evolution and even in our development from the egg. Genes seem to hold the stage just now, but it is not at all clear just how they induce development or evolution. The field is still open to speculation, one of the great sports of mankind.

SCIENTIFIC EVENTS

THE BRITISH NATIONAL PHYSICAL LABORATORY

THE annual report of the National Physical Laboratory, which appeared recently, according to a summary in the London *Times*, states that during 1934, the year under review, there was an increased demand for industrial investigations, which was most marked in the work called for by the shipbuilding industry. The much greater attention given throughout the country to the subject of noise was also reflected in the work of the laboratory, and there was an increase in the number of investigations.

At the William Froude Laboratory no fewer than 60 different designs of ships were tested, this being the highest number since the laboratory was opened in 1911. The modifications in design suggested and carried out by the laboratory have effected large improvement in connection with the resistance of a number of the vessels, and it is estimated that, assuming only one ship of each type was built, that each was steaming for only 200 days a year, and that the life of the ships was 20 years, the net saving to the industry in coal bills alone would be £500,000. Observations made of the height of waves in the Atlantic showed that in a storm they might be up to 25 feet high, rising to 40 feet in a hurricane and that the distance from crest to crest might be about 275 feet.

The subject of noise abatement received much attention in the new acoustics laboratory, and assistance was given to the Ministry of Health in connection with the sound-proof properties of modern walls for use in flats, to the Building Research Board on sound transmission through floors, and to the Ministry of Transport on the limitation of noise from mechanically propelled vehicles.

A new wind tunnel has been designed, and is now in operation, which can be used for studying the behavior of miniature aerofoils, a few inches in length, at a wind speed of 650 miles an hour.

The old British radium standard, which was prepared by the late Mme. Curie in 1913, has been replaced by a new standard consisting of a sample of radium chloride of higher purity. The British radium standard is used for determining, by comparison, the quantities of radium in the needles and other containers used by hospitals.

A section of the report dealing with road research states that special apparatus has been constructed at the laboratory for continuous measurement of impact forces while a vehicle is running along a road. Extensive tests are also being carried out with a heavy six-wheeled lorry running over rough and smooth roads near London, and over obstacles placed on the roads, and on a private road near Oxford impact forces at speeds up to 40 miles an hour are being observed. The results so far obtained show that the maximum impact causing damage to road and vehicle does not necessarily occur at the highest speeds.

Tests made with the object of enabling an aeroplane to fly stalled have led to the trial of a new biplane arrangement in which the upper wings are very much tapered while the lower wings slope considerably so that their tips come close behind the narrow tips of the upper wing. This arrangement was found to be as good as that of a normal biplane as regards performance, and to have a much higher degree of steadiness in stalled flight.

The report, a quarto volume of 260 pages, with 59 illustrations, is obtainable from H. M. Stationery Office for 13s. net.

EDUCATIONAL GEOLOGIC TRIPS IN PENNSYLVANIA

THE Pennsylvania Topographic and Geologic Survey in cooperation with the Pennsylvania Department of Public Instruction has recently inaugurated a plan for conducting geologic field trips for teachers and other interested Pennsylvanians. As a preliminary to the trips, some 2,000 copies of the Survey's bulletin 113, "Pennsylvania Geology Summarized," accompanied by a preliminary announcement of the trips, were distributed in March to high schools, normal