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OLD PROBLEMS AND A NEW TECHNIQUE[1]

IT is a truism to say that new instruments and new modes of technique may be as productive of advances in science as generalizations that point the way for many investigations. The telescope, the photographic plate and the spectroscope in astronomy, the chromometer and the thermometer in physics, the balance in chemistry, the microscope and the microtome in biology are trite examples of instruments and related technical processes so commonly used that their importance is forgotten. During the past twenty-five years, the string-galvanometer and methods of determining small amounts of gases in blood and other fluids of the body have been productive of great advances in physiology; while instruments for micro-dissection, and methods of staining, like those with hæmatoxylin, have made possible a flood of investigations.[2] The Greeks failed in

[1] Address of the retiring Vice-president and Chairman of Section F, American Association for the Advancement of Science, read at the Zoologists dinner, Nashville, December 29, 1927.

[2] The advent of such methods is sometimes vividly remembered by those who lived through the period. Recalling that I had once heard my former teacher, Professor S. F. Clarke, of Williams College, speak of the coming of ribbon sections in the early eighties of the last century and knowing that two other veteran American Zoologists, E. B. Wilson and E. L. Mark, were students abroad at this period, I inquired of these individuals. Each has contributed items of interest, but the statement by Professor Wilson is the most comprehensive. He says: "I first became acquainted with the ribbon method of section-cutting at the laboratory in Cambridge just after its discovery by Caldwell in 1882, and as I had very recently completed a research which involved the laborious cutting of great numbers of small eggs, one by one, and mounting the sections singly, you can imagine the surprise and pleasure with which I saw them reeled off wholesale by the ribbon method. As it happened I was, I think, the first person to introduce this method in Germany, having taken it over to Leuckart's laboratory in the early winter of the same year, and I have never forgotten the astonishment with which the operation was viewed by the group working there at the time. It was at first employed simply by the old method of cutting by hand with a razor on a flat-topped glass plate secured to a table, and was only a little later extended to the Thoma sliding microtome. It seemed to me then, and still seems to me, to be one of the most important steps

science as much through technical limitations as through their political collapse. In physical science, for example, they lacked accurate and convenient instruments for the recording of time and temperature, but more particularly they lacked methods of computation. One can appreciate the difficulties of such simple arithmetical processes as multiplication and division by Roman numerals, but the technique of the Roman system was less cumbersome than that used by the Greeks. The advent of the Arabic system, with its decimal point and with zero as a "place-holder," marked a new era for the natural sciences as well as for mathematics. I have thus referred to familiar advances of science through instruments and methods, because I believe the biological sciences have now at hand a technique that is not fully appreciated, although its importance has been demonstrated. I refer to the technique of *irradiation.*

The discovery of X-rays by Röntgen (1895) and of radium by the Curies (1898) was soon followed by applications in the medical field to diagnosis of gross features like skeletal fractures, and, when the "selective" action of the rays was discovered, to the treatment of malignant growths. That the more general possibilities in medicine were appreciated by Röntgen is shown by the fact that his original communication was made before a medical society and his first account published in a medical journal. These early applications were marked by sacrifice, since neither the danger to the operator nor the present modes of protection were known at the outset. Sterilization, burns, and even death were the lot of more than one radiologist, and patients no doubt suffered. But the present effectiveness of irradiation in the treatment of certain cancers is worth more than these earlier costs. Subsequent extensions of the method and of X-ray photography in clinical medicine are familiar. No hospital is complete, no physician's or dentist's service adequate unless X-ray diagnosis is available.

In correlation with the initial extensions in medical science, the interest of biologists was aroused, and during the first decade of the century certain general investigations were undertaken. Bohn ('03), Willcock ('04), and others conducted experiments upon the effects of radium and of X-rays in lower organisms. Gager ('08), exposed *Enothera* to radium and obtained changes that resembled mutations. The effects of irradiation upon the tissues and embryos of higher vertebrates were likewise examined in exploratory investigations. The "selective" action of the rays, in destroying certain types of cells, was studied by medical workers, and, later, the "differential" action of different wave-lengths. The existence of a "latent period" between irradiation and tissue changes was established. Within the cell, it was seen that the nucleus, and particularly the chromatin, was most susceptible to radium and to the X-radiations. There was, however, no such extension of radiology in the general biological field as occurred during the second decade of the century in the field of medicine. Interest in other lines, the war, and failure to obtain results that promised returns of general importance were no doubt responsible. While our medical colleagues went forward in the use of the X-rays in diagnosis and therapeutics, if not in research, biologists lagged behind. In like manner, ultra-violet irradiation began to be utilized in medical practice during the second decade of the century, without attaining a corresponding importance in biology.

My own interest in the technique of irradiation was aroused by a renewal of earlier studies upon the cellular changes during regeneration in planarians. In the regeneration of *Planaria maculata,* after reproduction by fission, it had been observed that cells called "formative cells" were the active units. These formative cells have relatively large nuclei and spindle-shaped cell bodies sometimes with extended processes. They divide by mitosis and seem capable of migration through the mesodermal syncytium or parenchyma. At the time of regeneration they are the only important source of new tissue. If my interpretations are correct, they form ectodermal, endodermal and mesodermal cells, nervous tissue, and special organs like eyes and pharynx. Moreover, they give rise to the germ cells when reproductive organs are formed. During the later stages of embryonic development similar cells are abundant in the mesodermal region. It seems, therefore, that the formative cells constitute a "meristem," which is the source of new cells during growth and regeneration, which has descended without modification from similar cells in the embryo, and which is the path of descent for the germ-plasm. These conclusions have been confirmed with minor exceptions by other investigators, and recently checked in *Planaria agilis,* another spe-

in the technique of microscopical anatomy, and especially of embryology. I do not know when the staining of sections on the slide was first introduced, but it certainly was prior, I think, to the discovery of the ribbon method; if I remember rightly it began with the use of shellac as the means of fixing sections to the slide, some time before the invention of the albumen method by Paul Mayer. It might interest you to know that I still have my old series of sections of the Renilla eggs cut singly, I think in 1881, forty-six years ago. I am obliged to confess that, from the modern point of view, they are rotten sections, but it cost blood to make them at the time.''

cies with powers of fission and regeneration similar to those of *P. maculata.*

Not all planarians regenerate as effectively as do *Planaria maculata* and *P. agilis.* In returning to the problem of regeneration during recent years, my interest was centered upon the relative abundance of formative cells in different species in relation to their respective powers of regeneration. It is a familiar fact to workers with planarians that some species have almost no power of regeneration. Such planarians are in marked contrast with species like *Planaria maculata* and *P. agilis,* which regenerate rapidly and completely. Other species of planarians have what one may call the "capacity" to regenerate, but only at a slower "rate." One may thus speak of the *power of regeneration* as including *capacity* and *rate.* A species like *Planaria maculata* or *P. agilis* has great capacity and a high rate, while *Dendrocœlum lacteum* has little capacity and a slow rate. *Phagocata gracilis* has the capacity, but the regeneration proceeds at a slower rate than it does in *Planaria agilis* or *P. maculata.*[3] To check these facts of general observation, experiments upon the relative powers of regeneration in these three genera (*Planaria, Phagocata* and *Dendrocœlum*) were undertaken, with results that confirmed what was known from more superficial examination.

With these differences in regenerative power established, the numbers and activities of the formative cells in the non-regenerating species, *Dendrocœlum lacteum,* were examined for comparison with *Planaria maculata* and *P. agilis.* This was done by making counts of these cells in measured areas. Although it was recognized that such counts could not be made without a large margin of error, it seemed that a quantitative statement might thus be obtained that would have more value than general terms like "few" and "many." As a result of such counts it was estimated that *Planaria maculata* possessed about 44 formative cells per unit area, and *Dendrocœlum lacteum* about 9, a difference that is in accordance with the powers of regeneration in the two species. It was therefore possible to make the declaration that the *power of regeneration is correlated with the number of formative cells.* If I may descend to a colloquialism—"show me the formative cells of a planarian and I will tell you approximately its power of regeneration, or show me its power of regeneration and I will tell you the relative numbers of its formative cells." The observations confirmed the conclusions regarding the rôle of formative cells in the regeneration of species like *P. maculata* and *P. agilis,* since it was shown that a species like *Dendrocœlum lacteum* with very few of these cells had scant power of regeneration.

At this point it seemed that confirmations might be obtained by further comparisons between regenerating and non-regenerating species, but that more direct evidence could not be obtained by the methods that had been employed. A more satisfactory test would be to destroy the formative cells and thus transform a planarian capable of regeneration into one incapable of such changes. The formative cells appeared to be undifferentiated and embryonic. If undifferentiated cells could be killed in higher animals by irradiation, it seemed that they might be killed by this means in planarians; and once they were killed it should be impossible for the individual to regenerate. Such experiments were tried with *Planaria agilis* (*cf.* Figs. 1–4). When a sufficient exposure was used (approximately 12 skin-units in clinical terminology) no regeneration occurred, although the worms remained alive without external signs of abnormality in structure or function for some time beyond the twelve-day period during which regeneration was completed in the controls of these experiments. When these irradiated worms were sectioned it was found that the formative cells were so reduced in numbers, if not all destroyed, that there seemed to be none remaining in many of the specimens. The mesodermal region was thus changed from its characteristic appearance in *Planaria agilis* to the appearance seen in *Dendrocœlum lacteum* in which there are few formative cells and in which there is scant power of regeneration. Lesser exposures to X-rays partially checked the regeneration and the numbers of formative cells were noticeably reduced. In an experiment with radium, in which an exposure about equivalent to that with the X-radiations was used, regeneration was inhibited in a similar manner.

Unfortunately, it has not been possible to keep the irradiated worms alive for an extended period. At the end of three weeks, or about one week after regeneration is complete in controls, the X-rayed individuals begin to show abnormalities of structure and function. Death ensues about four weeks after the irradiation. It is hoped that further experiments will give worms which can not regenerate, in which formative cells can not be found, and which can be kept alive in a normal state for longer periods. One could then eliminate the possibility that it is the destruction of something else in the planarian, upon which the formative cells are dependent, that is responsible for the check upon regeneration and that disappearance of the formative cells is merely the

[3] *Phagocata gracilis* is not so favorable for this purpose because of its multiple pharynges, but *Dendrocœlum lacteum* is a perfect illustration of scant power of regeneration as compared with a species like *Planaria maculata.*

Inhibition of Regeneration in Planarians by X-rays and Destruction of Formative Cells

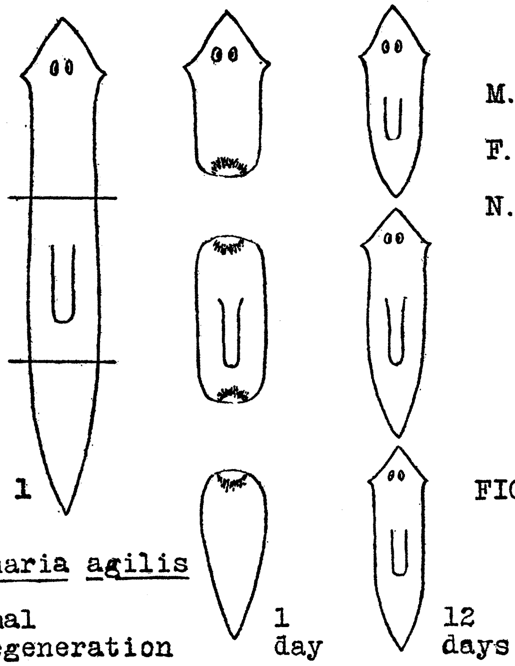

FIG. 1

Planaria agilis

Normal regeneration

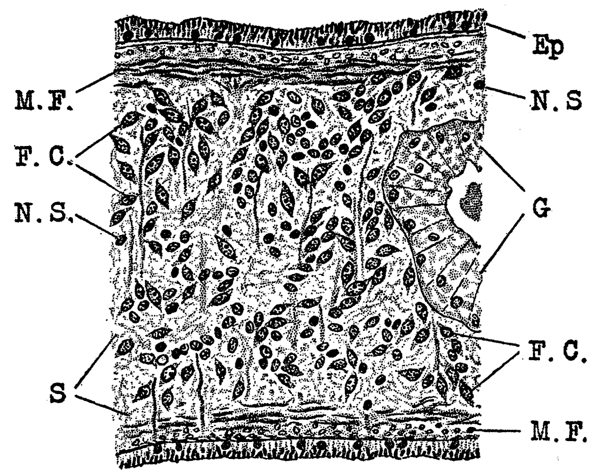

FIG. 3 Planaria agilis

Section showing the numerous formative cells in the mesodermal region of the normal worm

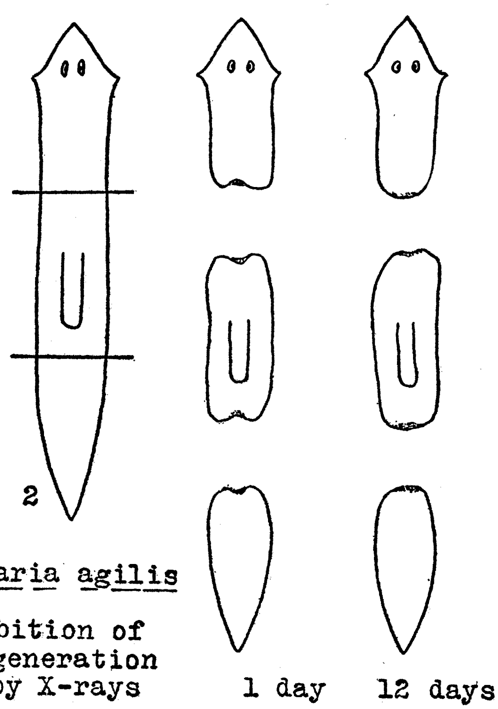

FIG. 2

Planaria agilis

Inhibition of regeneration by X-rays

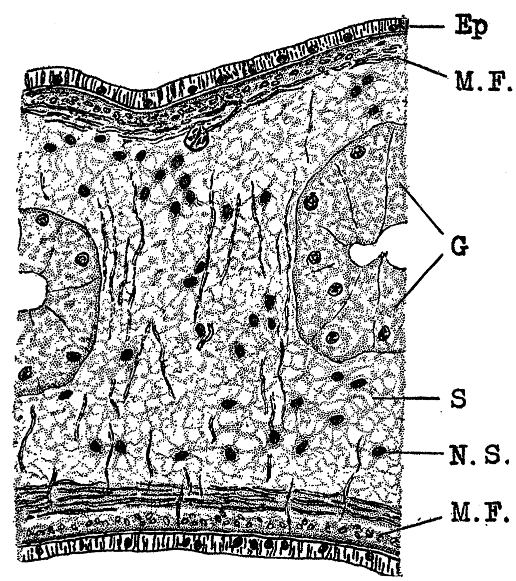

FIG. 4 Planaria agilis

Section showing destruction of formative cells by X-rays. The mesodermal syncytium, with its small, dense nuclei, the gut, and other structures are still normal in appearance

Ep, epithelium; F. C., formative cells; G, branch of gut; M. F., muscle fibers; N. S., nuclei of mesodermal syncytium; S, mesodermal syncytium. (Drawn by Wiley Crawford.)

first step in a series of degenerative changes that end in death of the individual. The formative cells may be the "indicators" and not the ultimate factors in regeneration. In view of our limited knowledge concerning the manner in which radiations produce their effects in protoplasm, this possibility must be considered. If one could secure irradiated planarians that could not regenerate and possessed no formative cells, but lived indefinitely with no other abnormalities in structure or functions, this possibility might be disregarded. According to the hypothesis that the new parts formed during growth and regeneration come only by differentiation of formative cells and that the germ cells of the planarian have a similar origin, such a planarian would be unable to reproduce either sexually or asexually and it would be incapable of growth by increase in the number of its cells. It would be an interesting animal. What would be its expectation of life? Would it exhibit senescence and rejuvenescence, if there are such phenomena in planarians? Would it eventually acquire new formative cells and become capable of regeneration? Would it have an axial gradient? A technique that gave such results would enable us to attack the problem of differentiation as it has not been attacked before.

In testing the formative-cell hypothesis as extended to other animals, experiments with X-rays have been conducted upon sponges and cœlenterates. It has been shown that regeneration can be completely inhibited in the hydroid *Tubularia crocea,* as reported at these meetings. An initial experiment with hydra has been likewise successful. The histological changes involved in these cases among the cœlenterates have not yet been ascertained. The experiments with sponges have not yet given significant results.

Proceeding to other illustrations of applications of the technique of irradiation: Regeneration is an aspect of development. What promise does such a technique hold for students of development in general? That much remains before we shall have reached a satisfactory understanding of ontogeny has been recently set forth in a masterful way by F. R. Lillie ('27), in an essay entitled "The Gene and the Ontogenetic Process." In speaking of the process of embryonic segregation, Lillie says: "We have no present working hypothesis of this most fundamental aspect of the life history." Workers in embryology, therefore, admit that they have reached a point of "diminishing returns" by sectioning and staining, by microdissection, and that even the talisman of physicochemical explanation has failed in its older methods. New technical methods are needed to carry us much farther and enable us to formulate new hypotheses.

There has been, as yet, no great progress, although enough has been accomplished to show possibilities by the technique of irradiation. Working with the ultraviolet radiations, Just has produced Nereis larvæ with nuclei that contain 70 chromosomes instead of the normal 28. Here the maturation divisions go on without polar-body formation so that the sperm nucleus unites with four egg nuclei instead of with one; each egg nucleus as well as that of the sperm has the haploid number of chromosomes—14, thus giving the total of 70. These larvæ produced by irradiation live in the laboratory as long as the controls. Ultra-violet radiations also change the original polarity of Nereis eggs and embryos. Eggs exposed before insemination show the site at which the egg is "hit" by the rays, since there is a blister at this place in the cortex. The first cleavage plane always passes through this area. As the eggs are not oriented for exposure to the rays, any region may be affected, the animal pole, vegetal, or any point in the infinite number between. Hence there is an alteration of polarity. The larval worms show a definitely localized area of injury which can be traced back to this original injury by the rays. The method of irradiation has also been used in various studies of artificial parthenogenesis and cross-activation that have sought to determine the rôle of maternal and paternal pronuclei and of the egg cytoplasm (*cf.* G. Hertwig, '13). While the results have not been revolutionary, it has been shown that irradiation furnishes a means of destroying parts of eggs, particularly the nucleus, in a way that can hardly be approached by mechanical operations. Moreover, it can be used effectively upon eggs *en masse* and without the toilsome procedure of operating with varying success upon each individual cell. In general, these results that have been obtained by irradiation as a technique of research within the field of embryology promise a method by which one may hope to destroy or derange parts of cells and embryos, without injury to the whole, in a manner that makes the finest mechanical operations crude by comparison. With such a technique, important advances may be expected in a renewed attack upon ontogenetic problems.

Turning to another field, Mavor, in studies conducted since 1920, has confirmed the selective action of X-rays upon undifferentiated tissues and upon germ cells in *Drosophila.* The resistance to lethal doses of the rays remains the same during the larval period; but increases markedly during pupation, when the adult parts are being differentiated, until it reaches a new level of stability in the imago. The most susceptible period in the germ-cell cycle is the growth period of primary oöcytes and spermatocytes in *Drosophila* as in other forms. Upon the basis of this underlying phenomenon of selective action by the rays, Mavor has produced non-disjunction in which the eggs either lacked the sex chromosome or possessed two sex chromosomes. Eggs having two sex chromosomes, when fertilized by a Y-bearing sperm, produced exceptional females which in turn produced exceptional daughters and thus propagated the condition brought about by the irradiation. There is, therefore, in this effect of X-rays on germ cells a very clear case of an external agent which modifies the mechanism of inheritance in such a way that a permanent effect is produced. In like manner Mavor has effected by use of X-radiations a reduction of the crossover value in the left half of the first or sex chromosome by X-rays, and an increase of the crossover value in the middle region of the second chromosome. He has also produced gynandromorphs from X-rayed mothers, presumably by elimination of the sex choromosome after fertilization. It is further interesting, as shown by X-raying the anterior and posterior halves of pupæ, that these effects upon the germ cells are not produced when a part of the fly not containing the germ cells is treated. That is, the effects probably occur only when the germ cells are subjected to irradiation and not through any influence transferred from irradiated body cells to the germ cells. It thus appears from the work of Mavor that X-radiations can modify the mechanism of inheritance, through chromosomal changes, in such a manner that permanent effects are produced and transmitted through successive generations without lethal abnormalities. The germ-plasm is changed, but not by the method of the Lamarckians.

The recent work of Muller upon *Drosophila* is widely known. Muller considers the changes he has produced by X-rays truly mutational and not to be confused with the well-known effects of the rays upon the distribution of the chromatin, like non-disjunction and non-inherited crossover modifications. Briefly, he finds that exposure of the sperm to relatively heavy doses of X-rays induces the occurrence of true "gene mutations" in a high proportion of the germ cells. The nature of the crosses was such as to be much more favorable for the detection of mutations in the X-chromosomes than in the other chromosomes, so that most of the mutant genes dealt with were sex-linked; there was, however, ample proof that mutations were occurring similarly throughout the chromatin. When the heaviest treatment was given to the sperm, about a seventh of the offspring that hatched and bred contained individually detectable mutations in their treated X-chromosome. Estimates of the mutations presumably produced in all the chromosomes indicate that the heavier irradiation had caused a rise of about fifteen thousand per cent. in the mutation rate over that in the untreated germ cells. Lighter irradiation produced the gene mutations in lesser numbers. "The visible mutations caused by raying were found to be similar, in their general characteristics, to those previously detected in non-rayed material in the extensive observations on visible mutations in *Drosophila* carried out by Bridges and others." Mutations already familiar, like "white eye," "miniature wing," and "forked bristles," were obtained, and also mutations of a sort not exactly like any seen previously. Muller, therefore, concludes that: "many of the changes produced by X-rays are of just the same kind as the 'gene mutations' which are obtained, with so much greater rarity, without such treatment, and which we believe furnish the building blocks for evolution." He also finds many inherited disturbances in the crossover frequency. In confirmation of these results, Hanson, who has been studying the effects of irradiation upon the sex-ratio of *Drosophila,* has obtained what he considers gene mutations as well as mutations that are seemingly due to chromosomal changes.

It thus appears that two types of genetic modification may be produced in *Drosophila* by the X-radiations—an aberrant distribution of chromosomes and hence new combinations of characters, and gene mutations. In confirmation of these findings in an animal, Stadler reports the production of both types of mutation in plants. In corn, irregular distribution of chromosomes occurs with a certain low frequency in the early cell divisions in endosperm development. This results in mosaic seeds, in which a portion of the endosperm lacks one or more chromosomes. The frequency of occurrence of this phenomenon has been increased about thirty-fold by X-ray treatment at the time of fertilization. Again, in young seedlings of barley Stadler has produced gene mutations by similar irradiation. In this instance the possibility that the gene mutations may in reality be the result of an irregular segregation has been eliminated. These mutations reported in barley are induced in somatic cells, from each of which a self-fertilizing inflorescence is later derived. Each mutant, therefore, segregates in the progeny of a single head, and its absence in the progeny of other heads of the same plant proves that the change occurred in the ontogeny of the plants irradiated. Several viable and morphologically distinct recessive types of such gene mutations have occurred in the progeny of the plants thus subjected to irradiation. To these studies by Mavor, Muller, and Stadler may be added the recent work of Gager and Blakeslee upon *Datura,* which shows irregularities of chromosomal behavior as a result of X-irradiation and which suggests gene mutations and other intrachromosomal changes. The results reported by Goodspeed and Olson upon tobacco plants seem to be in this category, since they do not clearly indicate gene mutations.

From the theoretical standpoint, the chief interest of such results within the field of genetics lies in their bearing upon the problem of the composition and behavior of chromosomes and genes, and thus upon the mechanism of heredity, variation, and evolution. From the practical standpoint, any technique that will induce abundant mutations presents opportunities for the production of improved breeds of domesticated animals and plants beyond anything dreamed of by the slow method of selecting what nature offers. To secure mutations that he desired, Burbank raised plants by the millions, discarding the ninety-and-nines. The advantage of more frequent mutations is obvious. On the medical side, these results, as Muller explains, point to dangers in the practice of temporary sterilization by irradiation. Return of fertility after irradiation does not mean that the germ cells are again normal, since mutations may have been induced that will appear in descendants with disastrous consequences.

Fully developed as well as latent genetic characters may be changed by irradiation. As part of a general program dealing with the biological effects of X-rays, Hance and Murphy have found that following certain exposures the colored hair of agouti mice drops out and after four or five weeks is replaced by white hair. Thus, in an adult animal, a character that is known to be inherited according to the Mendelian law can be changed by the X-radiations. The change seems permanent, as it has persisted for more than twelve months without modification. Speculating upon the causes of such an effect, Hance suspects destruction at its source of the enzyme tryosinase, the color activator, and hence failure of the oxidation of the color base, tryosin. At first what seem to be colorless tryosin granules are recognizable in the hairs, but later the hairs are without such granules and thus resemble the hairs of a normal albino. Nevertheless, "a permanent change has been produced in a mature genetic character by means of X-rays. It is believed that this change is the result of the inhibition or actual killing of the part of the cell concerned with the manufacture of the enzyme needed in the production of color. Since the new color, or rather lack of color, is permanent, it follows that the alteration is passed on to the cells that replace the ones that first encountered the X-rays."

A most interesting attack upon the entire problem of irradiation is being undertaken at the University of Cincinnati, under the leadership of Dean Herman Schneider and Professor George Sperti. The organization of this research is such that individuals in all the related fields, such as physics, chemistry, mathematics, bacteriology, zoology, and physiology, work in intimate association and with mutual support in a laboratory organized for basic investigations. By means of more accurate filters than have been previously used, these investigators have determined critical wave lengths at which chemical dissociation is produced in certain inorganic compounds, and in certain enzymes. They have also determined a wave length at which death occurs in many species of bacteria, and in a variety of the lower animals. Working with ultra-violet radiations, they find, for example, that all bacteria, thus far subject to investigation, are killed when a certain wave length (about 2900 A°) is reached. These particular results have been turned to practical advantage by patenting methods of sterilization for commercial products, the royalties from which are to be used in support of subsequent investigations. A specific example is that of an enzyme that was placed on the market for bread-making, but proved unusable because of bacterial contamination. To kill the bacteria by heat would have destroyed the enzyme, to kill them by antiseptics was not possible in a food product. When the problem was presented to the group at Cincinnati, the solution that suggested itself was to use a wave length that would kill the bacteria, but not destroy the enzyme, since it had been found that the lethal wave length differed in the two instances. The attempt was successful and I understand that the enzyme, minus the troublesome bacteria, is now in use.

To consider more general aspects of the problems open to attack by this new technique, one may distinguish between the obvious morphological and physiological effects produced by irradiation, in destruction of cells and modification of functions, and *how these effects are produced,* that is, the nature of the action of radiations upon protoplasm. The histological and cytological effects can usually be determined with no great difficulty. If one merely attempts destruction of certain parts in order to determine their rôle in development, as with the formative cells of planarians, one may leave the question of how these effects are brought about to investigators whose competence lies within the physico-chemical field. But the problem of how the changes are produced commands attention, because of its intrinsic interest and because it is likely to give clues to physiological processes that have eluded our understanding. This indeed may be the more important item in the end. Most investigators have used the methods of the morphologist. It is clear, however, that disturbances which effect changes that can be directly observed in cells are likely to be of a rather gross nature and that the more delicate functions of cells can not be adequately determined by such methods. Perhaps the studies upon the effects of irradiation in *Drosophila*, spectacular though they are from the standpoint of heredity, will prove more important as a means of detecting physiological changes, too subtle for other modes of demonstration, than as a means of changing genetic constitution. Such studies provide a means of analyzing the effects of X-rays on cells. They show how X-rays may produce a permanent change in a cell without altering its vitality. A method of experimentation has been developed indicating lines of further investigation which may help toward a clear understanding of the exact physical and chemical changes produced by X-rays in living protoplasm, and so of hidden physiological processes. This physiological aspect of the problem can not be elaborated within the limits of the present discussion, but it may be cited as an important field of investigation that is opened by the method of irradiation.

From the morphological standpoint, intensive study should now be directed to the changes produced within the cell. It was formerly believed that the cell was most sensitive during the period of mitosis. It still seems that chromatin is particularly susceptible to radiations, but it now appears that cells are most susceptible during the period just preceding mitosis. Thus, Mohr ('19) has shown, in his classic experiments with radium upon the locust, *Decticus*, that the primary spermatocytes and oöcytes are most sensitive during the growth period, although the cells may be easily affected during the maturation divisions. Working with cells from chick embryos under soft X-rays in tissue-cultures, Strangeways and Oakley ('23) have shown that the cell phases are affected in the following order: first the prophase; next, the later phase of mitosis; and last the inter-division phases of the cell cycle, although many of these "fully formed cells" remain normal in appearance even after prolonged exposure. These investigators have also demonstrated a latent period of about 15 to 20 minutes before the effects upon the cells can be recognized. It appears, however, that the changes produced by the radiations are not specific, since they resemble those in cells growing in unfavorable or in modified media. With lesser exposures, mitosis could be checked, but was resumed after a time if the cultures were returned to the incubator. Although the nucleus and particularly the chromosomes seem most sensitive to irradiation, the cytoplasm is also affected as Packard ('16) and others have shown. It even seems that one can distinguish between a primary effect upon the nucleus, as seen in the mitosis immediately following, and a secondary effect that occurs first in the cytoplasm although it is more apparent in the nuclear changes that it eventually produces (G. Hertwig, '20 and A. Politzer, '25). Between these two effects there may be a period of seemingly normal nuclear division (Stein, '26).

The study of physical and chemical changes within cells is subject to great limitations as compared with the study of such changes in non-living bodies. Methods of chemical analysis and synthesis have only limited applications, since they kill cells at the outset wherever they are applied effectively. Evidence from staining is subject to similar limitations. Microdissection supplements these methods, but has its own disadvantages. By such means, we "know in part and we prophesy in part" when we attempt "to prove all things" concerning cell activities. Results like those obtained by the group at Cincinnati, in destroying specific chemical compounds by specific wave lengths, suggest that it may be possible to destroy visible substances within nucleus or cytoplasm without lethal injury to the cell and thus identify their chemical nature. Such a method may lead to results undreamed of in our present philosophy of cell physiology. Consider what might be done with the problems related to the cytoplasm by such a technique. Results no less remarkable seem to have been accomplished in the nucleus without lethal injury to the cell.

We are thus confronted by the fundamental physiological problem, *the nature of the effects of radiations upon the cell*. Of this we know relatively little, although one can by irradiation change the nature of cells without lethal injury, as the geneticists have done; or completely destroy one type of cell without apparent injury to other types in the same organism, as in the familiar cancer therapy and as I have done in experiments with planarians. The only effect of the absorption of X-rays by an atom that physicists recognize is the expulsion of a high-speed electron. This must be the starting point in any purely physicochemical theory of the action of X-rays upon protoplasm. The problem is one for the biophysicist. If he can solve it, we may hope for notable advances in our knowledge of cellular physiology.

Problems that demand immediate investigation, if biologists would make full use of the X-rays and other radiations as an instrument of research, are: the exact measurement of exposures, the cause of the latent period, the "differential" action of diverse wave-lengths, and whether light exposures actually have the stimulating effects that have been claimed. Fields of biological investigation that seem most vulnerable to an immediate attack by this technique of irradiation are: morphogenesis, heredity, and the physiological effects of radiations.

Belief that the method of irradiation presents alluring possibilities as a tool of biological investigation therefore seems justified by results already accomplished. It is possible by use of radiations to destroy certain types of cells, as though by a surgical operation of surpassing delicacy. We can also reach within the cell and effect changes, particularly in the nucleus. It seems that we can even change the genes and thus inheritance. Most important of all, irradiation promises clues to basic physiological processes. In medicine it has found many applications. It may assume equal importance in the breeding of domesticated plants and animals. In the general field of biological science it offers a new technique before which old problems may fall.

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SPACES OF STATISTICS AND THEIR METRIZATION[1]

WE may generalize the ordinary statistical graph by considering an n-dimensional space in which each coordinate represents a statistical variate. How much arbitrariness is there in the choice of a particular coordinate system? In many familiar cases almost any change of coordinates is meaningless; geometry can then hope to throw but little light on the problem, compared to that which it supplies for physical problems, in which such transformations as those of the Euclidean group are held not to change the nature of the case. But certain situations arise in which real invariant properties exist for rather extended groups of transformations.

One of these cases is in economics. Let p_1, p_2, . . . , p_n be the prices of n commodities; and with these prices ruling, let q_1, q_2, . ., q_n be the respective quantities that can be sold. Now the same market situation can be expressed otherwise by giving the commodities different definitions; *e.g.,* a certain quantity of iron and wood will make a certain number of Fords and a certain number of garages; if we know the equations of transformation we have the same information given by either pair of quantities. Wheat of different grades can be, and is, mixed in different ways to meet the grading systems in different countries. We can readily prove that under these transformations in the manifold of prices, quantity is a covariant vector. Dually, in the manifold of quantities price is a covariant vector.

Another example is in biology. If an individual be regarded as fully specified by the dimensions of n organs he is, by definition, a point in a space of n dimensions. A species is a cluster of points, and may be typified by its center of gravity. Coordinates may be transformed by changing the methods of measurement; thus we may give the height of a man's shoulder and the length of his arm, or we may give the height of his hanging hand and the length of his arm.

But in all this no invariant distance element has appeared. We are impelled to look about for some quadratic form, preferably a quadratic differential form, of statistical significance.

Now a quadratic form of overshadowing importance is found in the exponent of the normal law of probability in n variables. The various reasons for adopting this law are strongest when the deviations of which the quadratic form is a function are small, and so it is natural to take it as a quadratic differential form.

The idea of random migration has received much mathematical consideration by Karl Pearson, Lord Rayleigh and others. It may be applied to particles in a biological n-space to discuss evolution. A son differs from his father in n ways; if these deviations are all independent and have equal dispersions, the probability of a set of deviations δx_1, . . . , δx_n is proportional to e^{-T}, where T is a constant multiple of $\delta x_1^2 + \delta x_2^2 + \dots + \delta x_n^2$, and so may be said to define the *distance* from father to son. If the deviations are not independent, product terms $\delta x_i \; \delta x_j$ appear in T. If finally the dispersions and degrees of interdependence of the δx's depend on the x's we have the quadratic differential form with variable coefficients, and therefore a Riemannian geometry. That the dispersions do in fact vary with the size is evident by considering the difference in centimeters in the length of a pair of twin elephants, and then comparing this with the variability in length in a litter of mice. It may safely be assumed that the intercorrelations, for a given species, may also vary with size and shape.

In this way we obtain a metrical space, in general curved, as a matrix for possible organisms. A species, represented by a swarm of particles, diffuses gradually by an accumulation of small changes in a manner analogous to the conduction of heat in this curved space. It would eventually become so diversified as to supply the naturalist with every conceivable kind of specimen were it not for the effect of selection. This may be pictured as a system of heat "sinks," of refrigerated localities, spread here and there to trap and annihilate unwary individuals. On the whole the losses due to the sinks are of course made good by natural increase.

The center of gravity of a swarm of individuals may be taken to represent the species. Suppose that we have a collection of fossils which shows us the location of this point at each of two times a million years apart; it is desired to know its most probable positions at intermediate times. If we have no

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