AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JANUARY 19

SOME CONSIDERATIONS REGARDING MICROSCOPICAL TECHNIQUE[1]

C. E. McCLUNG

From the Zoological Laboratory of the University of Pennsylvania

It has been said that the discovery of a new method may do more to advance science than the enunciation of a new principle. If this may, with any truth, be asserted of one method how really significant must be the comprehension of the technical aggregate upon which a science rests! Microscopical technique is variously regarded by biologists. To some it is an end in itself—a sufficient field for the exercise of all the powers of the investigator; to others it is a necessary evil to be endured only so far as it makes apparently satisfactory returns for the time spent. An exclusive acceptance of either position is a mistake leading to indifferent results. Technique is a tool, but an indispensable one, and, as yet, but imperfectly developed. It needs most careful study and merits all the care and attention we can devote to it. Progress in microscopic anatomy is largely dependent upon the refinement of present methods and the invention of new ones. Each serious student in this field of biology owes it to his chosen science to contribute something that is new or to suggest means for bettering the methods now in use. Rigid attention to all details of procedure is absolutely required in cytological investigations, and we may rightfully demand this of each worker as a prerequisite to the acceptance of his results. So much is the least we may ask—beyond this we can reasonably expect contributions to our present technical armamentarium. This expectation will be realized when the fundamental importance of the technical side of our work is appreciated and better means for its development are provided.

[1] An evening lecture delivered at the Marine Biological Laboratory, Woods Hole, July 27, 1917.

THE ANATOMICAL RECORD, VOL. 14, NO. 5
MAY, 1918

PROTOPLASMIC CONDITIONS

A consideration of the reaction between living protoplasm and chemical or physical agents must involve both members of the combination. Nothing is more obvious than the specificity of protoplasm, but changes which any particular variety may undergo are much less understood and appreciated. That there should be such a condition of variable reaction is no cause of surprise when the seasonal peculiarities of eggs under the same experimental conditions are recalled. Despite these facts there is little attention paid to the physiological condition of material submitted to the action of fixing fluids and stains, it being too generally assumed that the resulting differences in appearance are occasioned by the technique. Doubtless all experienced investigators can recall instances of inexplicable perversity on the part of apparently well understood materials and processes, resulting in such departures from the expected results as to raise grave doubts concerning the adequacy of our methods. Rarely, however, is the fault ascribed to the material and yet, as I hope to show, even the slightest modification of the protoplasmic element may markedly change the end result.

VALIDITY OF PHENOMENA

The importance of an exact knowledge of cell morphology increases from year to year as the conviction grows that in this structural unit are presented, in the most simple, available terms, the varied biological problems which engage our attention. In view of the fact that we rarely see a cell in its normal living condition the question becomes acutely serious regarding the adequacy of the phenomena we study in prepared material. This is a consideration to which thoughtful microscopists have given careful attention, and from many lines of evidence the conclusion has been reached that, in most respects, the product of our technique is representative of the living cell. It is hardly necessary to indicate the basis of this belief more than cursorily. Perhaps the most weighty evidence of this concordance is the high degree of uniformity in appearance obtained by the most

varied processes. Such variation as appears, in its major aspects, is clearly due to shrinkage, tearing, expansion or other physical changes which can reasonably be accounted for or predicted. Added to this is the close agreement in appearance between such details as are visible in the living cell and the same structures in fixed material. There is nowhere, in the variations under different systems of treatment, any suggestion of indefinite or unrestricted change, but only modifications of limited order involving a common series of elements everywhere recognizable.

SCOPE AND METHODS OF THE PROBLEM

While this uniformity exists, and is most encouraging, there is an accompanying variability in details which is often most annoying and puzzling. Where the purpose is to ascertain, down to the limit of vision, the exact architecture of the cell parts, this uncertainty of appearance may prevent the attainment of a confident judgment regarding some of the most significant cellular conditions. Since our effort should always be to reduce to their lowest limits the unknown variables in our problems the occurrence of these unpredictable changes in our materials is a constant challenge to the conscientious investigator.

So far our only method of attack has been largely through modifications of our empirical methods, but it has seemed possible to add to them some more reasonable means for determining the causes of our variables. Knowing the great interest all biologists must have in these fundamentals of our methods and realizing the insuperable difficulties in the way of the individual worker attacking alone the complexities of the problem, I am giving the results of a series of investigations upon microscopical technique, extending over a number of years, in the hope of contributing something to our common store of knowledge concerning the most trying, difficult and essential part of our work. In obtaining these results I have had the invaluable help of many of my students, among whom I am particularly indebted to Doctors Sutton, Carothers, Allen, Whiting and Hance. Without the thoughtful, efficient and discriminating help of Miss Ca-

rothers particularly it would have been impossible for me to have accomplished any large measure of the results so far obtained. Even with all this assistance my experience comprehends a relatively small range of materials and touches chiefly nuclear structures. Fortunately, from other sources, much information has been gained about cytosomic conditions, especially relating to mitochondria.

FRESHNESS OF MATERIAL

Some of the conditions involving protoplasm in its passage through technical treatment are fairly well understood and appreciated. The necessity for immediate fixation, for instance, appeals to most workers, but it is surprising to find the laxity which prevails even here. It is seriously advocated by some that a delay in fixation is desirable for the 'improvement' of the cell. Nothing is more certain however than that changes occurring after the death of the organism are, from the beginning, destructive. There is a possibility that some of these may favor the demonstration of certain structural details, but the inherent chance of error is so great that results obtained by this method could receive credence only when confirmed by more reasonable means. The extent to which post-mortem changes occur in a given period vary much with the nature of the material and the attendant physical conditions. In general there is a progressive liquefaction of the protoplasmic gels which eventually manifests itself in the prepared objects by vacuolization and loss of fine detail.

AGE OF SPECIMEN

In plant cells the changes incident to age are clearly marked and do not escape notice, but in animals these are less obvious and often fail to receive consideration. It is true that cleavage phenomena have been studied and the relation of nucleus to cytosome determined, together with the distribution of certain physically distinguishable substances, and Minot has given us a con-

ception of the nucleo-cytosome relation in adult cells, but we are yet lacking an understanding of the variations which must be ascribed to the age conditions of our prepared material. Without having made any careful study of this subject I have come to realize certain conditions in Orthopteran germ cells which must be due to the age of the organism. Among these are a greater density of the protoplasm, more of a tendency toward degeneration, and less precision in response to reagents. Hartman believes that there is an increase in size of the chromosomes with age. This is a subject which should receive careful attention.

METABOLIC CONDITION OF THE ORGANISM

At any given period in the life history of an organism the reaction of its cells to physical agents varies with metabolic conditions. This fact has been demonstrated in our experience with Orthopteran cells through changes resulting from the use of different food plants and through the invasion of the body by fungi. If Chortophaga is fed upon grass its reaction to fixation in Flemming, and to staining in haematoxylin, is such as to produce a pure nuclear stain after appropriate handling. Other animals of the same species, fed upon clover and subjected to the same technical treatment, show a reversed staining reaction. A similar reversal occurs in the cells of animals whose bodies are invaded by the mycelia of a fungus which not infrequently attacks grasshoppers. So profound is the change thus produced that it is practically impossible to secure a normal nuclear stain with haematoxylin.

METHOD OF KILLING

At first thought it would seem absurd to consider that the method of killing an animal would have any effect upon its cells, and yet it has been clearly proven that certain well marked differences obtain between the cytological details of animals killed by cyanide or by xylol. Ever since the beginning of my work

upon the Orthoptera I have been familiar with two contrasting conditions in the first spermatocyte metaphase. In one case the spindle is long and clean, the chromosomes extended and well distributed, and the cytoplasm clear and bright. Under the other condition the spindle is short and restricted, the chromosomes are contracted and crowded, and the cytoplasm is granular and hazy. Notwithstanding numerous efforts to produce these appearances at will by modifications of the different steps in the technical processes they remained unaccounted for. By exclusion and fortunate circumstance it was finally determined that killing the animal by dropping xylol upon it would eventuate in the extended spindle while animals killed by cyanide fumes would show cells with the contracted spindle. Experiments now under way have shown that like changes may follow other killing agents.

It has of course long been recognized that various animals require very careful treatment before they are subjected to the action of killing and fixing fluids. Unless they are narcotized they contract so strongly that their cells are useless for study. Some marine flat worms reduce themselves almost to the state of unorganized jelly under the action of ordinary fixing agents. That the relatively stable cells of the grasshopper could be influenced by the almost instantaneous process of killing did not occur to me, and in this respect I do not find myself alone, judging by the lack of published observations on this point. After the discovery of the actual facts and a careful consideration of the case the variations thus produced did not, after all, seem so strange. An animal, after death by cyanide, is limp and flaccid and there is no tendency for the jumping legs to break off. On the contrary, an animal killed by xylol is stiff and rigid and the jumping legs are either cast off or easily break from their attachment. If these lethal agents so differently affect the muscular and connective tissue cells it is not at all strange that other cellular complexes, including the germ cells, should manifest differential results. Whether the action upon the muscle fibers is the same as upon the spindle fibers is another matter,

but it would not be strange to find it so. Although experiments have not yet been carried far they indicate that the action of anaesthetics, like chloroform and ether, is similar to that of xylol, while decapitation produces the same result as cyanide. It is possible that the disturbances resulting from decapitation are due to alterations in the pressure of the body fluids but we do not have any definite information upon the final cause of any of these changes.

METHOD OF TREATMENT IN FIXING

While it is generally admitted that the tissue must be so exposed to the action of the fixative that ready penetration can be effected it is not so clearly appreciated that slight variations in the conditions existing at the time of fixation can produce extensive differences in the finished product. One of the most striking instances of this appears when the same form of material is treated with the same fixative, in one case being in a reasonable sized mass and in the other a smeared film. A fixative which gives atrocious results in the former case may produce good smear preparations. My experience has been that, with Orthopteran cells, almost any fixative will produce a good smear preparation. It must not be assumed that this is because no fixing agent is required, for this material, unlike the Hemipteran, cannot be fixed by drying but requires the immediate application of a fixative.

The size of the mass alone will not determine the character of fixation—immediate penetration and ready exchange of fluids must be provided for. In the study of Culex cells, as has been described by Whiting, Hance and Holt, although what appeared to be a good preservation was obtained by removing the abdomen and fixing the gonads *in situ*, when a careful study of the chromosomes was made this was found to be very poor. Indeed this method seemed to indicate that the diploid chromosome number is three, as has been asserted by two European observers, when, as a matter of fact, it is six. This is easily demonstrated by removing the gonad, freeing it of adherent fat and fixing in Flemming. Under these conditions the paired elements remain

entirely separate and distinct, whereas without these precautions the members fuse together and the number of chromosomes is apparently one half what it really is. In Drosophila the first spermatocyte metaphases are rarely seen unless the testis is freed of fat and trachea.

Similar difficulties arise in the treatment of the mammalian testis where clumping of the chromosomes invariably results unless the seminiferous tubules are teased out or otherwise exposed to the direct action of the fixative, as has been shown by Allen and Hance. It may safely be said that almost all the work done on mammals is worthless largely because of neglect at this period in the technical process. The more fluid the chromosomes are the greater the difficulty in preserving them as separate and distinct entities. Under faulty treatment they tend strongly to flow together, with apparent numerical reduction, and this may go so far as to show metaphase plates as flattened masses of chromatin with occasional openings through it. Even a casual inspection of published figures will show the prevalence of this condition.

LENGTH OF FIXATION

It is not enough to choose an appropriate fixing agent and to apply it properly—the time of its action is important. This varies with the character of the tissue, its size and with the fixing agent used. A familiar result with most cytologists is the glassy and more or less homogeneous appearance of Amphibian testis cells treated with osmic mixtures. The same kind of cells may be immersed in picro-formal-acetic mixtures for an indefinite period without harm, while grasshopper cells exposed to osmic mixtures for days will not be injured. On the other hand if the period of immersion be too brief the preservation is incomplete and faulty. Drosophila spermatocytes in metaphase, if given insufficient treatment with Flemming, are reduced to amorphous masses.

CHARACTER OF FIXATIVE

Of all the steps in the technical processes that involving the character of the fixative has received most adequate attention. It is generally recognized that the agent must be adapted to the material, and emphasis can not be too strongly laid upon the importance of this fact. At the same time, as can be seen by reference to other statements I have made, the character of a fixation can not be judged properly unless it is known how the material has been affected by other agents in the process. It is also true that some combinations are, under proper conditions, generally applicable to a large range of materials, and this is more true of all fixatives than has been realized. Combinations of acetic acid, picric acid and formalin may be said to constitute a universal fixative because they can be applied to so many kinds of materials to preserve the finest details of structure without detrimental effects. It is however necessary to realize that even with accurate preservation of structures their full demonstration may require a special fixation. Thus while the picro-formal-acetic mixture may show the presence of mitochondrial structures they can not be made evident by specific stains. It is therefore required that we not only choose an appropriate fixative for the type of cell we wish to study but we must also make the selection in view of the particular cell structure to be investigated. Having decided upon the substances required it yet remains to be determined in what relative strengths they should be combined and with what adjuvants. To do this properly an extensive and time consuming series of experiments is necessary and the advantage of cooperation becomes apparent. It is possible that after a number of such series has been completed some better general principles will be developed to guide new investigations.

Such a cooperative and extended study of the action of certain chemical fixatives has been undertaken at the University of Pennsylvania by Doctors Carothers, Allen, Whiting and Hance with very encouraging results. The details of these have been published in part and will not be repeated here, but it should be noted that the relative strengths of the different members

of the combination are important. It also appears evident that the presence of certain adjuvants contributes materially to the perfection of action in any given case. Thus the addition of urea to the picro-formal-acetic mixtures or to Flemming produces well marked and characteristic effects upon both plant and animal cells. Various sugars and malic acid correspondingly improve the action of fixatives upon plant cells, each producing a distinctive reaction. In general the action of these inert substances is to preserve the more fluid parts of the nucleus, so that otherwise empty spaces are shown in sections to be filled with a delicate reticulum. Increased concentration or specific action may however result in vacuolization of the cytoplasm. The great advantage of these additions to the fixing fluid for chromosome studies is in preventing the somewhat liquid chromatin of certain cells from flowing together with the consequent loss of chromosome outlines.

PHYSICAL CONDITIONS DURING FIXATION

Little attention, relatively, has been paid to the physical conditions under which the protoplasm and its coagulant are brought together, and yet this is far from being an unimportant matter. Temperature, concentration of the reagents, method of application, the presence of adjuvants and many other circumstances are significant. I wish here to speak particularly of the relation of temperature to the fixation process. The optimum conditions appear to vary both with the material and with the fixative. It seems generally true that for mammal tissues a temperature of 0°C. with Flemming, and 38°C. with picro-formal-acetic is best. There seems to be less difference in the case of invertebrates but this is dependent somewhat upon the rapidity of penetration. Our experience so far would seem to indicate that if immediate action is secured, as in a film, the temperature is not important, but that if time is required to bring the cell and reagent together it should be considered. The explanation for the variation in temperature required to produce equally good results would seem to be that a low temperature holds the tissue

unchanged during the period required for the penetration of a slowly moving reagent, while the higher temperature raises the rate of penetration of the fixative which is thus brought to the cell before it can undergo alteration. Of course with so high a temperature as 100°C. the action is directly that of the heat.

WASHING

Once the protoplasm is coagulated by fixation we are then dealing with a solid instead of with a semi-fluid substance, but this does not signify that the object can be treated with any less care. In fact many of the poor preparations which we see are the result of neglect in after-fixation stages. Without exaggeration it can be said that the best of fixed material may be spoiled at almost any period of its subsequent treatment. I should like to give some examples of these changes which have appeared during the course of our experiments, beginning with the next step of washing out the unused fixative from the cell. According to all directions this should be thorough, but apparently the principal object sought is to remove an extraneous substance for physical reasons. It seems certain, however, that marked differences in a fundamental staining reaction may follow in tissues which would commonly be regarded as well washed, due to the further extraction of chemical substances. Haematoxylin, while not a test for chromatin, nevertheless, under ordinary conditions, gives a pure nuclear stain with sufficient extraction. This was the universal result in all my own Flemming preparations, but every year when students in my cytology class made slides from fresh material they secured a reversed staining reaction, the nucleus being unstained and the cytoplasmic structures darkly tinged. Finally the yellow color of the nucleus in these latter cases, together with the amount of color in the alcohol which had stood for some months on my own unsectioned material, suggested that possibly insufficient washing was responsible for the reversal of stain. To test this out paraffin sections were made of recently fixed material, which had been washed as usual for twenty-four hours, and the ribbon

spread and dried. The paraffin was removed with xylol from the sections at one end of the slide and left protecting the other half. Thus prepared the slide was soaked in seventy per cent alcohol for three days, after which the paraffin was dissolved from the remaining sections and the whole slide stained in iron-haematoxylin. Upon differentiation a very peculiar slide resulted, one end showing cells with a pure nuclear stain, the other exhibiting excellent mitochondria but no stained chromatin. The length of washing is thus shown to be the means of securing a mitochondrial stain or a chromatic stain with the same material and the same reagents. With other fixatives no such results might follow and, in some, washing can be omitted completely. This part of the procedure must be dictated by the character of the material, the nature of the fixative and the object sought.

DEHYDRATING

In dehydrating extensive shrinkage, distortion and tearing may result. The gross differences thus produced have been considered by Allen in a recent paper, where it is stated that the major portion of shrinkage in the whole series of technical processes comes during dehydration. Besides these more general effects upon the whole tissue there are possible local changes within the cell which are significant. There has been extended discussion of the phenomenon of synizesis, much of it being fruitless and uncalled for. In an early paper I stated that this condition in the Orthoptera is an artifact and suggested that possibly it might be of similar character in other cases. This incidental remark has been more quoted and discussed than many of my most important statements. In this case synizesis is purely an artifact resulting from the too sudden passage of the material from one fluid to another. It never occurs in properly dehydrated material and, while it may not always result from sudden dehydration there is the possibility of it, which should be avoided by gradual removal of water. Aside from this question of synizesis, which probably is not nearly so important as it has seemed to some, there are many other changes produced by alcohol which need not be discussed here.

CLEARING

Removal of alcohol by some agent miscible with it, on one hand, and with paraffin on the other, may be attended with some of the same difficulties connected with dehydration, which can be avoided in large part by the proper selection of the agent and by its careful application. Shrinkage and hardening are the worst evils which follow the use of clearing agents. Some years ago I tested the shrinkage action of various substances upon young chick embryos and found that while some of them reduced the dimensions of the disc almost a half, others caused a contraction of less than ten per cent. The least injurious of these was cinnamic aldehyde following eighty-five per cent alcohol. Allen reports that aniline oil, after seventy-five per cent alcohol, produces no appreciable diminution in volume even after twenty-four hours' action. While we have no exact data on the subject it seems certain that the gradual transition from alcohol to the clearing fluid results in distinct advantages.

INFILTRATION

One of the worst difficulties in the paraffin method is the shrinkage and distortion which comes from the application of the hot paraffin. So severe is this action that the method is entirely inapplicable after certain fixatives. I have already referred to the fact that good smears may be secured by almost any fixation, but material fixed in Vejdovský's chrome-sublimate or with Helly's modification of Zenker suffers such contraction in the hot paraffin as to be useless for study. That this shrinkage and distortion occurs during infiltration was demonstrated to me by Doctor Danchakoff who carried some of the same Zenker-formal material through the celloidin process with excellent results. Doubtless much condemnation of fixing methods is unjust because the other circumstances attending the technique have not been properly appraised. There are some tissues also which can not be carried through the paraffin method because they are either too hard or too soft to withstand its action.

SECTIONING

During the process of sectioning changes of great moment may occur, many of which are familiar to all experienced workers. I need not speak of such effects as compression, tearing, splitting, folding and cracking of paraffin sections because they are obvious to all. Less marked, but of great importance in chromosome studies, is the removal entire of chromosomes which are either lost or carried over into neighboring cells to cause double confusion. Extreme care is needed in sectioning—the knife must be perfectly sharp, it must be inclined to the plane of the section at exactly the right angle, the paraffin should be homogeneous and of a density suited to the material, and the room temperature must be adapted to the thickness of the sections required. The resulting ribbon should consist of sections little less in diameter than the face of the block from which they were cut—straight, free from scratches or breaks and of uniform thickness. Such can be obtained only by the most exacting care in detail but are essential to good results.

SMEARING

If the smear method is employed it should be carefully checked by a study of sections. Properly used it is a valuable method but there are many difficulties attending it. Some material, like blood or the germ cells of the Hemiptera, may be distributed over a glass surface and fixed by drying, but the same procedure applied to Orthopteran cells results in a featureless expanse of unorganized material. Such cells must be distributed between two covers with exactly the right pressure, the covers separated just so and the films fixed at once. The covers should be of the correct diameter for the amount of material between them and the pressure applied adapted to the density of the cells of that particular species. With the utmost care the cell elements suffer various distortions which must be recognized by comparison with sections. Some cells can not be smeared at all because of their fluid character and it is not uncommon in the best smears to find apparent multinucleate cells resulting from the fusion of sepa-

rate cells before fixation. It is almost literally a fact that one may find nearly any condition imaginable in a smear. The need for great caution is therefore clearly manifest.

STAINING

Time will not suffice for any adequate consideration of the process of staining. The number of conditions to be demonstrated, the variety of the staining agents available and the complexities of the processes are too great for anything less than a monographic treatment. I wish merely to call attention to the adequacy of two or three well understood stains for all general purposes. Iron-haematoxylin alone will serve to demonstrate almost all nuclear and cytoplasmic structures which can be specifically distinguished by other agents. If to the use of this is added the safranin—gentian violet—orange G. combination the cytologist is prepared for almost any condition aside from the most special studies.

MOUNTING

It would seem to be the opinion of some cytologists, judging by their preparations, that once the sections are obtained any method of getting them on the glass will do. And yet, just at this point the greatest care is needed if any size comparisons are to be made. A complete and uniform spreading of the ribbon is absolutely required and no pains should be spared to secure it. I do not hesitate to say that unless this step is properly taken conclusions regarding size and form of cell structures are almost worthless. A matter of apparent small importance is the straightness of the ribbons on the slide, but this mole hill may well become a mountain of difficulty in an extended study and materially reduce the effectiveness of the observer. A little time expended during the preparation of the slide will save much, later, and may even be the determining factor between good and bad results.

CRITERIA OF JUDGMENT

It may seem somewhat aside from a discussion of technical problems to consider the subject of criteria of judgment in micro-

scopic anatomy, and yet it is so intimately a part of the whole problem of interpretation involved that I can not refrain from speaking of this most essential and neglected phase of the work. Under the microscope things are not what they seem—every image produced by the lenses must be interpreted in the light of the observer's experience. The microscopical appearance of a sphere of air in water is about as widely removed from that of the actual object as could well be imagined. Many cellular structures require the same translation by experience when studied microscopically. The literature of chromosome studies alone is filled with false interpretations and worthless theories because of the neglect of this elementary conception of microscopical images. In most instances we study colored images, but these are superimposed upon refraction images and the distinction is not always realized, with the result that there are descriptions of longitudinally split rods in which the split is really a refraction line or of divided threads which are only hollow tubes. Even more obvious errors than these are frequently made. A glance at the figures of metaphase chromosomes in many papers will show them to vary in diameter strongly and to have pointed ends. Neither of these conditions normally obtains and their representation is due to the fact that the observer neglected to note that these apparent variations are due to a failure to focus each region at its optical section. Thus the fundamental organization of the chromosome is misrepresented because the observer has not realized that a knowledge of the third dimension of his object must be gained by focusing the microscope. If such an elementary knowledge of microscopical conditions appears lacking in an observer's work the experienced investigator at once suspects graver defects and discounts both the observations and the conclusions. No one can afford to place himself in an unfavorable light at the very beginning of his presentation by the neglect of the fundamentals of the science.

CONDITIONS OF MICROSCOPICAL OBSERVATION

There are many considerations involving the choice and use of the microscope and its accessories, the manipulation of the

light and the substage condenser, the comparison of images produced by high and low magnification, etc., which are of the utmost importance in exact microscopical observation, but which I have no time to discuss here. I can not refrain from saying, however, that every microscopist should understand thoroughly the instrument upon which he depends. Without such an understanding his results are sure to lack precision and accuracy.

THE PERSONAL FACTOR

There is one matter upon which I should like to speak, but hesitate to do so for fear of being misunderstood. Since it is one of such fundamental importance however I shall venture to express myself freely, trusting to the good judgment of my readers to take what I say in the spirit of helpfulness in which it is intended. An experience, which I am surprised to find extending to twenty years, has taught me that cytologists, like poets and other specialized individuals, are born to their work and can with difficulty, or not at all, be diverted into it. There are certain qualities, the possession of which is no warrant for undue self esteem, that are demanded of the person who would devote himself to the investigation of the intricacies of cellular phenomena. Without these, disappointment is bound to come to the individual and trouble to his fellow workers. The literature is burdened with papers which were at best doomed to a fruitless existence because their authors were not qualified by nature or training for the work which they undertook. In full recognition that this same statement may with truth be made of many other lines of endeavor, I would still say that it is particularly true of the exacting work of cytological observations. It does not follow even that because a man is a good histologist or embryologist that he will make a successful cytologist. There is no other type of work with which I am familiar that calls into play so strongly the qualities of infinite patience and care, the nicety of manipulation, the exactness of observation, the discrimination of values, the judgment of relations, the exercise of good common sense together with the need of well balanced con-

structive imagination, as does the finest cytological work. Before any person should decide upon the career of a cytological investigator he ought to demonstrate to some experienced worker the possession of the necessary qualifications for it. Even then, only time and a long suffering scientific public will be able to tell whether in his case the eminent cytologist was as good a judge of men as of cells.