THURSDAY, FEBRUARY 22, 1900.

A NEW DEPARTURE IN CYTOLOGY.

Fixierung, Färbung, und Bau des Protoplasmas. Kritische untersuchungen ueber Technik und Theorie in der neueren Zellforschung. Von Dr. Alfred Fischer, a.o. Prof. der Botanik in Leipzig. Mit einer coloristen Tafel, und 21 abbildungen im text. Pp. x + 362. (Jena : Gustav Fischer, 1899.)

IT is many years since Dr. Routh administered to a youthful aspirant after literary fame the celebrated advice to "verify your references," and no matter what be the branch of learning concerned, the maxim applies, *mutatis mutandis*, with equal force to all. Certainly no one devoted to science can afford to disregard it, since extracts from the difficult text of nature are hardly less liable to errors of transcription than to those of interpretation. And this is especially true in the case of a study such as that of cytology, for not only is the evidence difficult to obtain and the degree of its relevancy to a particular problem hard to decide, but its very authenticity is not to be admitted without full proof.

Prof. Fischer has thoroughly deserved the gratitude of all who are interested in the investigation of cell phenomena, for he has opened up (even if he cannot claim to have originated) a new and fruitful path of inquiry in attacking the very foundations on which our knowledge of cell structure is based. He has, in fact, conducted an extensive investigation into the reactions obtaining between certain well-known proteids (in the widest sense of the term) and the fixatives and stains which are in common laboratory use.

The importance of this procedure becomes at once apparent on reflecting that protoplasm, when killed, becomes in fact something else, yielding, *inter alia*, a complex mixture of proteids, and that it is just these proteids which are so grouped in the dead cell as to produce what we term its structure.

Many of the proteids can be tolerably easily recognised and classified, and it is clearly imperative to learn as much as possible about them and their individual reactions with the substances employed to kill or fix the cell contents. It may be regarded as fairly certain that "fixation" really implies the *precipitation* of substances which, in the living cell, existed under other forms and other conditions. In some cases, *e.g.* when salts of the heavy metals are used, it is probable that the metallic base enters into combination with some, at any rate, of the proteids, whilst in the case of some other fixatives, such a possibility appears to be excluded. But in whatever way the precipitates may be produced, it is obviously a matter of prime necessity to ascertain the extent to which they correspond, in position and in form, with the structural elements of the living cell. An instance in which such a correspondence is probable is supplied by the chromosomes of a dividing nucleus. These bodies can be identified in the living condition, although it is not until after fixing that the finer details of structure become apparent, and for which it would be hopeless to look in the living nucleus. Now it is precisely as to the extent to which we are justified in regarding these

details as faithfully reflecting *living* structure that the greatest uncertainty prevails.

Many persons are satisfied by the comparative uniformity exhibited in "well-fixed" specimens when prepared by diverse methods. But can the criterion of trustworthiness be safely sought in exquisite differentiation alone, even though a multitude of reagents should conspire to produce it, unless the specific effects of each fixative be thoroughly understood? Perhaps few reasonably cautious cytologists, thus pressed, would return an immediately affirmative answer, although they could hardly conceal from themselves the fact that it is too often on the tacit admission of some such postulate that many of the current theories and hypotheses depend. Small wonder then if these should sometimes prove untenable when the soundness of their foundations has been insufficiently tested.

Prof. Fischer devotes the first seventy-two pages of his book to the consideration of the reactions of certain selected proteids (*e.g.* albumen, albumose, peptone, nuclein) with the reagents commonly employed for killing and fixing the cell contents. The amount of detail is almost bewildering, and, indeed, throughout the book the reader seeks in vain for short and clear summaries of the many lines of argument.

The results obtained are such as to throw considerable light on the operations of killing and fixing. Some reagents, *e.g.* osmic acid, although they rapidly destroy life, do not precipitate the proteids; and this fact explains why cells killed with osmic acid retain a considerable resemblance to the living condition. But the action of osmic acid in this respect is easily affected by the presence of other substances, *e.g.* acids, in the cell, whereby further changes may be induced. Other reagents, such as acetic acid or alcohol, bring down some of the proteids, the rest being left in solution; whilst, finally, there are still others, for example, chromic acid or mercuric chloride, which have a far greater fixing (*i.e.* precipitating) power.

Again, the nature of the precipitate is often characteristic: witness the coagulum produced in albumen, nuclein, or nucleic acid by treatment with mercuric chloride; whereas, if chromic acid be employed instead, nucleic acid and albumose are both precipitated in a granular form. But the final result is also subject to some amount of modification, depending on the conditions of the experiment. Thus alkalinity, acidity, excess of either reagent, may all affect it, though in a way which can be calculated and reckoned with. A consideration of no little importance attaches to the fact that, in mixtures of proteids, the individual precipitate-form characteristic of each proteid is still retained when they are thrown down by a fixative, and the bearing of this upon the probability of arriving at a correct understanding of the structure of living protoplasm, from a study of fixed preparations, becomes immediately obvious. Indeed, it is extremely probable that much of the so-called "structure" may turn out to be the mere expression of chemical idiosyncrasy—interaction between proteid and reagent—rather than an instantaneous photograph, so to speak, of the true structure of protoplasm. An example will make this clear. Altmann's solution (consisting of potassium bichromate and osmic acid) produces a very constant appearance in protoplasm, consisting chiefly of

well-defined granules lying in a spongy framework. But a closely similar image can also be produced by substituting a mixture of serum-albumen and deuto-albumose for the protoplasm, and it is at least open to suspicion that the appearances are *evoked* in the protoplasmic corpse, as they certainly are in the mixture, by the definite action of the chemicals employed.

But, although it may be conceded that the study of fixed (and stained) preparations is, after all, largely one of the structure and relations of precipitates, it would be going a deal too far to regard this as at once invalidating the results which have been arrived at by a careful use of histological methods. Thus in many cases it will readily be admitted that the precipitation-figures indicate and correspond with regions of active and special metabolism within the cell. It may well be, however, that we shall find ourselves on safer as well as on more fruitful ground in investigating the nature of the substances involved in cellular activity rather than in studying the minute details of structure. Some, indeed, have already regarded as illusory many of these appearances which now would seem to have been summoned into existence by the mere act of coagulation or precipitation. Zacharias, as well as Fischer, though working on different lines, has shown how much valuable knowledge can be obtained by micro-chemical methods, and these are still in their infancy.

After dealing with fixatives, the author next turns his attention to staining, and in particular to the effects of the aniline dyes. It is obviously impossible to discuss the whole of this complex subject here. Those who are interested in the matter may turn to Prof. Fischer's treatise itself, where they will find an immense supply of further information and facts.

The author arrives at the conclusion that a chemical (in the ordinary acceptance of the term) interaction between dye and tissue elements is out of the question, and that the process of staining is a purely physical one depending on the "adsorption" or condensation of the dye on the surface of the ultimate particles (micellæ) of the stainable substance. It is pointed out that acid dyes differ in important physical characters, such as solubility, from the basic stains; and it is argued that these differences, coupled with the physical structure of the body to be stained, will account for the particular colour reaction produced in any given instance. These conclusions will come as a shock to those who have been accustomed to pin their faith on the aniline dyes, and who have seen in "Cyanophily" or "Erythrophily" an index of chemical distinction between the various groups of cell-constituents. The evidence brought forward by Dr. Fischer in support of his thesis is based not only on the observations and practice of technical workers; he has himself contributed an elaborate series of experiments designed to elucidate the phenomena of dyeing; and if his results are not always free from ambiguity, some margin, as he himself hints, must be left as a tribute to our imperfect acquaintance with the objects and processes concerned. Nevertheless, so far as the positive evidence goes, it certainly seems to afford confirmation of the physical, rather than the chemical, theory of staining phenomena.

Following the same plan as that which yielded good results in his investigations on fixatives, the author ex-

perimented with precipitates of known proteids. He finds that no constant relations exist between the staining capacity of a proteid and its power of forming a precipitate on adding the dye to its solution. Thus nucleic acid, which takes up basic, but not at all acid, dyes, is only precipitated from solutions by the former. On the other hand, albumen, whilst also only precipitated by basic dyes, is stained more especially by acid ones.

It is very well known that it is essential in the case of certain fixatives (*e.g.* those containing chromic acid or platinum salts) to carefully "wash out" the superfluous fixative before staining is possible at all. Prof. Fischer uses these facts to support his views, assuming that just as the stain may be adsorbed, so also, in some cases, the fixative may be attracted and held, leaving no place for the stain until it is thoroughly washed away—a process which is often attended with considerable difficulty. The same peculiarity of inhibiting staining is shared by some other substances, though they may form no precipitate with the dyes employed; thus tannin, which precipitates basic, but not acid, dyes, impartially inhibits the staining power of them all in proteids saturated with it. Again, although platinum chloride precipitates eosin but not acid-fuchsin, a proteid fixed with this reagent and not subsequently washed will not take up either dye; whilst chromic acid, if it be substituted for the platinum salt, although behaving in precisely the same way towards *solutions of these dyes*, does not hinder their staining at all.

As might, perhaps, have been anticipated from the author's own standpoint, the fixative used may modify the primary capacity for stains possessed by the proteid, but this secondary effect is probably to be connected with a changed molecular or micellar form.

Another argument against a chemical explanation is founded on the behaviour of precipitates of even the same proteid towards mixtures of stains. Precipitates of albumose, for example, which contain granules of various sizes, select the stain according to their bulk, the larger granules taking up and retaining the acid (more rapidly diffusible) dye, whilst the smaller ones are coloured by the basic stain. There seems no reason to suppose that the granules differ chemically *inter se*, although this objection has actually been raised in other quarters, and Fischer interprets the effect as being due to the difference in solubility, concentration, and rate of diffusibility of the two classes of stains, coupled with the degree of tenacity with which, when once adsorbed, the molecules of dye are retained on the micellæ of the proteid. The reversals of stain so frequently met with under these conditions appear also to tell in favour of the physical or mechanical hypothesis. But the author by no means confines himself to a study of simple proteids, he also discusses the bearings of his results on the staining reactions of protoplasm itself.

Amongst the more interesting of the latter may be reckoned the characteristic differences exhibited by the nucleus of the male and female gametes respectively. Dr. Fischer regards the cyanophily of the male as due to the dense state of aggregation of its substance, and not at all as indicative of a chemical dissimilarity between it and the erythrophil female nucleus. His case is not weakened by the fact that, in those instances where

fusion of the two nuclei is delayed, the male pro-nucleus as it grows and gradually assumes a less dense appearance, approximates more and more in its staining reaction to that of the female pro-nucleus. Nevertheless, it might also be argued that the very state of dense aggregation implies a chemical difference, especially when it is recollected that growth perhaps involves much more than mere expansion of bulk.

But it must not be supposed that the evidence adduced is everywhere immediately favourable to the author's standpoint. There are, in fact, many anomalies, especially in the case of certain basic dyes, which require to be cleared up before Dr. Fischer's views can command universal acceptance. It is, however, as has been already said, impossible to do justice to this part of his book within the limits of a single article. But as the facts adduced will be largely new to most cytologists, it has been thought best to utilise the available space in pointing out a few lines of the argument rather than, by venturing on detailed criticism, to trespass on the domain of the chemist or physicist.

By no means the least interesting part of the book is that devoted to an inquiry into the origin of cell-structure, spindle-fibres, centrosomes, and the like. As regards the spindle-radiations, the results of some remarkable experiments are detailed, and these are quite startling owing to the apparent fidelity with which certain of the most characteristic features of cellular activity have been successfully imitated. Other investigators have produced, ere now, appearances resembling the achromatic spindle, but the figures have never attracted serious attention, as the analogies between them and a protoplasmic structure appeared to be too feeble. In the present case, however, it is very different, since the materials employed are akin to those which exist in the cell.

By injecting elder-pith with sundry colloids (albumen, albumose, &c.), and then treating sections of the pith with various fixatives, the author succeeded in obtaining spindle structures closely resembling those exhibited in a preparation showing karyokinetic figures. Not only this, but it has been found possible to ascertain at least some of the conditions which are necessary to their formation.

In the first place, there must be present in the cell some foreign indifferent body which may serve as a centre from which the radiations may start. Such a body is often provided in the dead but persistent fragment of the original cell-nucleus. Secondly, the precipitating action of the reagent must be slower than its penetrative power—it must have completely saturated the fixable substance as far as to the foreign body, before the physical change of precipitation sets in. Unless these conditions are both complied with, no radiations will arise. The importance of the second point at once becomes apparent when one recollects the emphasis laid on *rapidity of penetration* as one of the essentials of a good "fixative," and an analogy between the first condition and the origin of spindle fibres is seen in those multipolar spindles associated with large heterogeneous bodies in the cell protoplasm, which were first pointed out by the present writer in 1893, and which have since been confirmed by Mottier and many

other investigators. Similar results were also obtained by allowing fixatives to diffuse from capillary tubes into the fixable proteids; the radiations then started from the surface of the concentrated drop of fixative in such a way as to simulate the appearance of an attraction sphere.

The author exercises an admirable restraint in instituting comparisons between these results and those observed in preparations of cells, but it must not be forgotten that there is reason to believe that substances very similar to, if not identical with, those employed by him do really exist at least in the killed cell.

Naturally much is still left obscure. Why, it may be asked, should the precipitates only take the form of radiations during mitosis? If we attempt to frame an answer temporarily satisfactory on chemical or even microchemical grounds, we should have to trek into regions far outlying the limits of our present knowledge.

A discussion of cytological phenomena and of the various theories of protoplasmic structure and its supposed mechanism, occupies the remainder of the volume. The centrosome in particular comes in for a lengthy criticism, especially as regards those alleged for the higher plants. The author, on good grounds, concludes that these are merely cast out nucleoli, or, at any rate, in no sense to be regarded as special cell organs. He points out the fallacies which have led to the obscuring of the true issues in the past, and he treats the well-known case of *Lilium* with special severity.

It will be apparent that Prof. Fischer's book is not only startling from the novelty of its contents, but it is even almost revolutionary in its tendencies. But the cytologist need not be unduly alarmed, nor fancy that all his tenderly nurtured theories must of necessity dissolve forthwith into vapour. Probably much of the "structure," which was believed in by the extreme adherents of the particulate school may turn out to be due to *post-mortem* effects, and to possess no counterpart during the life of the cell; but the broad distinctions of cytological structure will still hold good, even though they may not be able to support the weight of the theories that have been erected upon them. The chromosomes, the spindle-fibres, the centrospheres (where apparent) all represent definite facts of protoplasmic activity, although the conclusions which have been drawn respecting them may stand in need of revision. But it is well that we have been thus recalled to examine once again, and more minutely than heretofore, the very foundations on which our knowledge of cell phenomena rest. *Diligenter explorata principia ponantur.*

J. B. FARMER.

RESEARCH IN PREVENTIVE MEDICINE.

Transactions of the Jenner Institute of Preventive Medicine (late British Institute of Preventive Medicine). Second Series. Edited by Allan Macfadyen, M.D., B.Sc., Director. Pp. xv + 253. (London: Macmillan and Co., Ltd. New York: the Macmillan Company 1899.)

DURING the last year the Jenner Institute of Preventive Medicine has passed through a most notable phase of evolution; with it has become associated the name of the father of modern preventive medicine, whilst its sphere of usefulness has been