First report on the cytological investigation of cancer : 1906 / by J.E.S. Moore and C.E. Walker.

UNIVERSITY OF LIVERPOOL AND ROYAL INFIRMARY
CANCER RESEARCH LABORATORIES

(Mrs. SUTTON TIMMIS MEMORIAL FUND).

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DIRECTOR

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WITH ILLUSTRATIONS AND APPENDICES

PUBLISHED FOR

THE LIVERPOOL CANCER RESEARCH COMMITTEE

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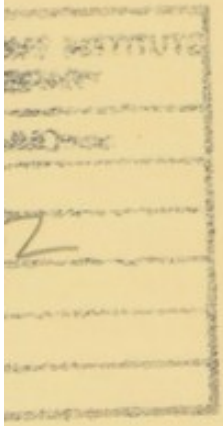

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PREFACE.

The investigations, an account of which is contained in the present publication, have been carried out in the Johnston Laboratories, a part of which was generously placed at the disposal of the Cancer Research Committee by the University of Liverpool. A considerable increase in the working staff of the laboratories has been made during the current year. This has been made possible through the generosity of Mr. T. Sutton Timmis, who made a substantial addition to his original donation. The thanks of the Committee are due to the University not only for the space in which to carry on the work, but also for the allotment of the particular rooms in question. Through this the researches have had the inestimable advantage of being in close contact with other kindred departments of the University, and with the Royal Infirmary.

In the latter institution a room was lent to the Committee wherein material from the wards could be treated in any manner that was desirable, and this has been of immense advantage to the work at different times.

The thanks of those concerned in the Research are especially due to Sir Rubert Boyce, F.R.S., Professor Sherrington, F.R.S., Professor Benjamin Moore, Professor Ross, C.B., F.R.S., Professor William Carter, Sir James Barr, Professor Rushton Parker, and Mr. Paul.

Lastly, the thanks of the Committee and ourselves are due to Professor J. B. Farmer, F.R.S., who as joint author of several of the papers referred to in the sequel, did much to originate the special line of enquiry that has been pursued. To Professor Farmer we are also indebted for much help and kindly criticism in relation to several papers published in the proceedings of the Royal Society and elsewhere within the last few months. The researches in relation to cancer which have been pursued in the laboratories, have followed upon cytological work begun several years ago in the Biological Department of the Royal College of Science, London. These earlier researches were connected with reduction phenomena in animals and plants, and especially with the constancy and similarity of this process throughout the animal and vegetable worlds.*

The appreciation of the cyclical character of the growth of reproductive cells, led us to enquire whether the abnormal cell-developments occurring in cancer were not connected with the reappearance of characters belonging to the reproductive cycle in those somatic tissues which, under ordinary circumstances, never exhibit them.

From a comparison of the cytological characters of numerous tumours we were led in the first place to conclude that some of the phases of the reproductive cycle are present among cancer cells. In the second, that these peculiar characters could not be demonstrated in growths of a purely benign character.

The results of these investigations were published in a preliminary note read before the Royal Society in 1903. The position taken up in this note was subsequently greatly

* "The Maiotic Phase (Reduction Division) in Animals and Plants," 'Quart. Journ. Micro. Sci.,' vol. 48, 1904. Farmer and Moore.

strengthened by the ascertainment of the fact that the bodies known as the archoplasmic vesicles and found universally as a striking characteristic of mammalian reproductive cells, could be directly compared with the so-called "Plimmer's bodies" encountered within the cells of cancerous growths. In dealing with this matter we have been greatly indebted to Dr. Plimmer himself, who most kindly placed at our disposal many of his own preparations and much information respecting the bodies with which we were concerned.

The results of this comparison were communicated in a preliminary note published in the Proceedings of the Royal Society, 1905.

At the close of the last year, in conjunction with Professor Farmer, we read before the Royal Society a paper containing further investigations concerning the cytology of malignant growths. In this the reduction phenomena as occurring in cancer were dealt with in a more extended form. The effect of these latter investigations has been to show that with respect to the existence of heterotype divisions and reductions of the chromosomes among cancer cells, our original position was correct. In the details of the evidence concerning this subject, however, the reader is referred to the later pages of the present report.

Our communication referred to in the preceding paragraph was the last published in conjunction with Professor Farmer, but since the work has been transferred to Liverpool, independent communications have been issued from the department as follows:—

(I.) "The Maiotic Process in Mammalia." Moore and Walker. 'Thompson-Yates Reports,' 1906.

(II.) "On the Synapsis in Amphibia." Moore and Embleton. 'Proc. R.S.,' 1906.

(III.) "On the Existence of Permanent forms among the chromosomes in the first Maiotic division in certain animals." Moore and Arnold. 'Proc. R.S.,' 1906.

(IV.) "Observations on the Life History of Leucocytes." C. E. Walker. 'Proc. R.S.,' 1906.

(V.) "On the origin of the Sertoli or Foot-cells of the testis." Walker and Embleton. 'Proc. R.S.,' 1906.

In view of the fact that several separate publications have been issued at one time and another in connection with the Liverpool Cancer Research, it has been thought advisable that all these memoirs should now be reprinted for reference, together with the present general account of the investigations. In what follows, however, owing to the unfamiliar character of the matters dealt with, it has been found convenient to preface the special account with a description of the Maiotic phenomena and their significance in relation to questions of general biology.

The thanks of the Committee are lastly due to the Editors of the 'Thomson-Yates Reports.' The Council of the Royal Society, and the Council of the Pathological Society, for their kindness in allowing the various memoirs mentioned above and others to be reprinted in the present report.

CONTENTS.

PART I.

MAIOTIC PHENOMENA, CELL DIVISIONS, AND GROWTH.

1. All enquiries relating to the life and growth of living things resolve themselves ultimately into cell problems, all vital processes being manifestations of the activity, growth and multiplication of living cells. In itself a cell may be described as the simplest protoplasmic arrangement that can lead an independent existence. A cell is constituted morphologically of a nucleus and its associated cytoplasm. These protoplasmic units may be separate from one another as in the protozoa, or they may remain in connection as in our own bodies. In the latter case they may be divided from one another by partitions, or they may be merely differentiated as independent centres of influence in a continuous protoplasmic mass. Neither the cytoplasm nor the nuclei of cells are simple substances, but even under the microscope are found to be visibly complex jellies, built up in the manner of foam, or the structure of a coarse colloid as it is understood by the modern chemist. Simple cells when immersed in suitable media, grow by converting the constituents of surrounding fluids into their own substance. Although the exact *modus operandi* of the process of growth is not understood, the act of growing is generally accompanied by a most remarkable phenomenon. While it is proceeding, each cellular unit divides like a drop into two drops, and these distinct processes of growth and division may go on indefinitely. When first observed the process of division was supposed to be analogous to the division of a simple drop into two, but the recent advances in our knowledge of the process have shown it to be of astonishing order and complexity. When a cell is at rest it appears to be a coarsely colloid jelly, within which there is a spheroidal area of distinct substance of the same nature—the nucleus. When the cell is about to divide the nucleus breaks up into a number of discreet parts composed generally of short band like bodies consisting of a dense material in which there is a variable amount of a substance—chromatin—that stains intensely with aniline dyes. These bodies—the chromosomes—lie within the nuclear area in a less stainable fluid. The most remarkable fact about them is that their number is constant for any particular animal or plant. In the cells composing the body of a man there are 32, in those of a donkey 36, and so on.

2. The fact that the number of the chromosomes is constant in the bodies of animals and plants is most remarkable, and its interest has been still further increased through the investigations of Flemming, Strasburger, van Beneden and many others, who showed that in every normal cell division, each of the chromosomes splits from end to end, and the halves so produced pass to the daughter cells as the whole mass divides. In this way when a cell divides we have in each daughter element the same number of half chromosomes as there were whole chromosomes in the parent cell. These half-chromosomes, collecting together in each of the daughter cells, become surrounded by a membrane, and swell up to form the same kind of spheroidal body as the nucleus of the parent cell, before the process of division began.

3. The constant number of the chromosomes appearing in each division has long suggested to cytologists that, though these bodies cannot be distinguished in most cases during the resting condition of the cell, they nevertheless must be present, or their number

could not remain the same. This conception of the permanency of the chromosomes has recently been greatly strengthened by a number of observations published by ourselves and others. Thus in the case of the cockroach *(Periplaneta)*, it is found that the chromosomes can actually be seen during rest. The same phenomenon has also been observed in the cells of *Triton*, as well as in cells of plants.*

4. In this way it would appear that the surmise of the older workers was correct, and it may be taken now as practically demonstrated that the chromosomes are bodies, that remain within the cells in approximately constant number. The only cell structure which really divides after the manner of a drop is the cytoplasm which surrounds the nucleus, and is dominated by it. Each cell is therefore in reality a very highly complex entity, and the complexity of the nucleus, with which we have just been concerned, is by no means the measure of cellular complexity as a whole. In the great majority of cells, extra nuclear bodies of constant appearance and relationship may be demonstrated by appropriate staining. They appear whenever the cells divide, though they may sometimes be seen in the resting condition. They have been described by Flemming, van Beneden, and many others, and are known as centrosomes. They are minute particles on the extreme limits of microscopic vision, generally paired and usually bean-shaped, one being larger than the other. When a cell divides the beginning of division is often first marked by the activity of the centrosomes. These bodies become surrounded by protoplasmic radiations, and separating from one another, take up positions in relation to these radiations exactly simulating the poles in a magnetic field. When the chromosomes have become fully formed, the nuclear membrane is broken up, and the chromosomes themselves become individually attracted by the centrosomes. In this way the two halves into which each chromosome is split become separated from one another and drawn towards the centrosomes at either end of the cell. When the chromosome halves have been completely withdrawn from each other, and lie in two separate groups at opposite ends of the cell, the activity of the centrosome wanes, the radiations disappear, and the whole cell mass gradually rounds itself off into two daughter moieties in the same way as any other viscuous drop. In reviewing the essential constituents of the cell, we must in the first place take into account the cytoplasm. In the second place we have the centrosomes, small definite bodies, generally lying in the cytoplasm. Thirdly a definite number of chromosomes, constituting the only permanent structural basis of the nucleus.

5. All these permanent constituents of cells, the cytoplasm, the centrosomes and the chromosomes, have each individually the capacity of dividing as they grow, and their conjoint periodical action in this respect brings about the complex phenomenon of cell-division as we see it.

6. It is a very important fact that, when a cell has divided, all the three sets of structures above alluded to have been directly derived from a fission of their pre-existing representatives in the parent cell. The daughter elements do not arise through a differentiation of the cytoplasm, of the centrosomes, or of the nuclear constituents, these three structures in every daughter element are portions of similar entities pre-existing in the parent cell from which

* Farmer and Moore. 'Quart. Journ. Micro. Sci.,' vol. 48.
Farmer and Shove. 'Quart. Journ. Micro. Sci.,' vol. 48.
Overton, J. B. Miyake, K. Strasburger, E. 'Jahrl für wiss. Botanik,' vol. 42.

the daughter cells have been derived. To put the matter in another way, during cell-division there is an accurate halving of every permanent constituent of the cell and the collection of these structures into two exact miniatures of the parent. No cells are known which possess less than the structural differentiation just alluded to, nor are there any composed of parts differing essentially from the above.*

7. The cell is to be regarded as the protoplasmic unit, because all living things, whether plants or animals, are made up of one or more cells, all having the structural relations just considered.

8. The gross animal and vegetable forms are now viewed as the result of the operation of natural selection, and it is a most curious and significant fact that directly we descend to cell structure the potency of natural selection as a modifying influence seems to come to an abrupt end. It has never succeeded in eradicating cell structure from a single animal or plant, and if—as most biologists would be inclined to believe—cell structure itself is a product of natural selection, operating in the past upon protoplasm which did not possess it, why are there no classes of living things which do not exhibit cell structure, their protoplasmic arrangement having been evolved along some other line?

9. It is suggested that cell structure lies as it were below natural selection, or in other words that cell structure is one of the ineradicable molecular concomitants of living substance.†

10. The protoplasmic units—cells—have the capacity to grow, and this capacity is, as we have seen, not comprehended at present as the result of any known physio-chemical activities. They have also the capacity to divide, which in like manner is a process we cannot corelate with growth or explain in relation to any known properties of inanimate material. The process of growth and the process of division may go on quite indefinitely. Thus we may graft a plant, and from the sprout graft again, and so on for ever. It seems probable that several trees, which are all of one sex and are propagated by cuttings, are living examples of grafts of centuries standing. In this way the vitality of tumour grafts which has so much astonished recent investigators, is nothing more than a universal and long recognized capacity in cells.‡

11. It was first clearly shown by Weismann that when we are dealing with unicellular organisms, death as ordinarily understood among the higher forms does not naturally occur. The organisms in question go on dividing without end, and their living representatives are simply the division products of the original cell from which they sprang.

12. In the higher animals, death occurs periodically, and the race is continued by means of cells which become liberated from the adult organism. Although under certain circumstances growth and division appear to be unlimited, under others these processes appear to terminate naturally together. The significance of this has been illustrated by the researches of Maupas, Hertwig and others. Dealing with numbers of ciliated infusoria they found that these unicellular animals would, if properly supplied with food, divide for a prolonged period, until

* In many of the higher plants, no centrosomes have hitherto been demonstrated. It may be, however, that they are multiple, and below the range of vision, or that the higher plants have lost them. The latter supposition is supported by the fact that the lower plants possess them.

† The monera described by Häckel as of the nature of Rhizopods without nuclei, have never been re-discovered.

‡ Bashford, Murray and Cramer' "Scientific Reports on the investigations of the Imperial Cancer Research Fund, No. 2, Part II., 1905."

a great number of individuals were produced. But after a time this process of multiplication comes to an end notwithstanding the fact that the supply of food has been kept up. In other words the dividing capacity of the swarm dies away, and when this occurs the individual protozoa conjugate in pairs.

13. After separating the "rejuvenated" gametes again proceed through a similar chain of "fissiparous bipartitions." This alternation of periods of division and conjugation goes on without limit. When, however, the individuals of a single swarm have ceased to divide, if they are prevented from conjugating, it is found that the members of the whole swarm degenerate and eventually die out. These highly interesting facts were first brought into prominence by Maupas and subsequently studied by Hertwig and others with similar results.

14. We thus see that, in the protozoa used in the above experiments, when the conditions remain constant and the supply of food is kept up, continuous growth and division comes to an end, and is succeeded by conjugation, which is again followed by divisions and growth. In these cases we have an alternation of the phase of division with that of conjugation, and these two phases succeed one another periodically and apparently without limit. The results we have considered with regard to the ciliated infusoria are in no way confined to that particular group of protozoa.

A similar alternation in phase has been discerned in all the other groups of unicellular organisms that have been sufficently investigated, whether animals or plants. This alternation bears an obvious relation to the sexual phenomena in the higher animals and plants, although it appears to differ from it owing to somatic death being absent among the unicellular forms. This difference is, however, apparent, not real, and we fully agree with Hickson and others that among the ciliata there are probably structures that actually correspond to the soma of the higher forms, *i.e.*, their macro-nuclei, which disintegrate after each conjugational act.

The difference between the uni and multicellular organisms is produced chiefly by the difference of association of the corresponding protoplasmic elements of which they are both formed.

The first may and often do consist at all times of a single cell. The second, in their adult form, are always great congeries of such cells. The unicellular organism is one of a more or less numerous crowd of cells arising through the division of some pre-existing element. The multicellular organism is a corresponding crowd, the individual members of which, however, are bound together so as to form a more or less elaborate soma.

15. The protozoon, like all its brethren of the same ancestry, is capable of dividing and conjugating. The greater number of the cells of the metazoon can divide, but do not usually conjugate. In them the latter function is relegated to a few special elements. The protozoon is therefore a single structural unit of protoplasm. The multicellular organism is a complex polity of such elements. In the metazoan fraternity all the component cells are different and perform different functions.

16. In a protozoan swarm the individuals arise from a division of a single ancestor after conjugation. In a multicellular organism the members of the cell polity arise in the same way. A multicellular body would thus correspond to a protozoan swarm if the products of segmentation remained distinct. It differs from the protozoan swarm firstly in that a large number of the products of segmentation become built up into a coordinate mass of organic machinery.

17. In the second place the cells composing this machinery, although they may divide and grow, do not tend to conjugate. The somatic organization eventually breaks up and disappears. At the same time these distinctions are not so great as they may appear, for in the somatic machinery produced by the segmentation of the egg of a multicellular form, there always remain some cells which do not belong to the machinery of the body in its adult form. From their first differentiation these cells, as it were, use the soma as a temporary asylum, and they retain a capacity, not only to divide and grow, but also to conjugate with other cells of a like nature. These, the reproductive cells of the multicellular organism, possess a life cycle precisely parallel to that of the members of a protozoan swarm. Besides the reproductive elements, there are other cells performing special functions within the machinery of the body, which are not structurally welded into the somatic organization. In most animals there are elements early separated off from the primitive epiblast, or hypoblast, or both, which afterwards lead an independent life among the spaces and fluids of the rest of the bodily machine. These cells form the great class of leucocytes, and they continually multiply, while as the organism becomes senile, they show no tendency to follow suit, but, as the researches of Metschnikoff and others have shown, continue active to the last, often eventually preying upon the worn out and enfeebled cell tracts of the decaying soma.

18. It is a remarkable and interesting circumstance that among the sponges, a group of organisms wherein the somatic differentiation, although complex, is curiously loosely held together, it is from amongst the leucocytes, or wandering cells, that the actual reproductive elements are finally produced.

19. Taking all these facts into consideration we see that it would not be correct to say that the multicellular organism consists entirely of somatic machinery *plus* the reproductive elements. As a matter of fact we find even among the higher vertebrates that there is a large class of elements which, like the reproductive cells, remain permanently outside the organization of the soma.

20. We have noticed previously that it was the reproductive cells in the multicellular organism which, in their capacity to conjugate and start new life cycles, paralleled the life history of the individuals of a protozoan swarm. We find in the porifera that both amæbocytes and reproductive cells spring from the same blastomeral stock. We are consequently led to enquire whether, in the higher animals some, or all, or any of the wandering elements cannot exhibit the phases of the reproductive cycle. The investigations of one of us upon this matter seem to show that they can.* These observations are again borne out by what we have ascertained respecting the development of cancer.† For the moment, however, it is necessary to introduce other considerations affecting our conception of the relation of multi and unicellular forms.

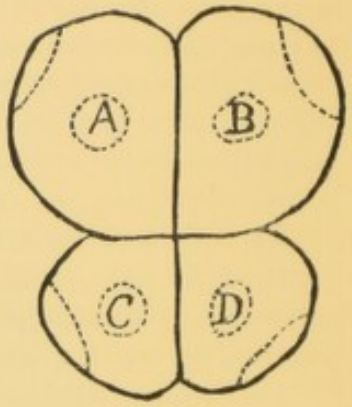
* C. E. Walker. "Observations on the Life History of Leucocytes." 'Proc. R.S.,' 1906. *See Appendix.*

† Farmer, Moore and Walker. "On the behaviour of Leucocytes in Malignant growths." 'Trans. Path. Soc., London,' vol. 56, part III., 1905. *See Appendix.*

COORDINATION.

21. We have seen that the multicellular organisms differ from the unicellular forms in that a great part of the division products arising after conjugation become definitely built up into the somatic machinery. During their conversion they become widely altered both in appearance and functions, and at the same time becomes mutually affected by the body influence of the species to which they belong. Their growth, division, and activities are henceforth strictly limited and also guided by some power long before the appearance of the central nervous system, or any nervous system at all.

22. If the blastomeres of an echinoderm larva in the two-cell stage are dissociated, each grows into a complete but small embryo. Thus each blastomere, like the fragments of plants, has the capacity to form the whole. The different blastomeres in the embryo (fig. 1) will give rise to structures having different attributes. If the blastomeres were shaken apart each would give rise to all the different tissues which would have been produced by all four had they been left together. In association A exhibits none of the potentialities of B, C or D. Out of association it possesses the potentialities of all of them Consequently the presence of B, C and D confine A to the manifestation of particular attributes. It is the same with each of the others, B, C and D in turn. The lens of an amphibian's eye is an epiblastic structure. If it be excised, a new lens may be formed, not from the epiblast, but from the subjacent mesoblast.



23. It has been shown by experiment that if an embryo be bisected, the lost half may be regenerated by the remaining half expanding as if the space occupied by the missing half were already there mapped out, and a heterogeneous assembly of cells simply poured into it.*

24. The influences which bring about these phenomena are at present quite obscure, but nevertheless they are of the very first importance. For the sake of convenience we shall speak of them collectively as coordination. Many attempts have been made to render the action of coordination intelligible. It has been found that most cells in plants are really in organic contact, or in other words that between cell and cell there exist minute protoplasmic strands. The same protoplasmic bridges are known to exist in the cases of numerous animal tissues. Assuming that among all the cells of a soma there exists direct protoplasmic continuity it has been sought to explain the phenomena of coordination by the further assumption that impulses of which we know little are passing along these channels about which we know less, and by their operation, the effects of which cannot be verified, the results of coordination may be brought about.

25. Another line of explanation has been sought. There are other instances of organic coordination, not among cells, but among a number of animals, namely human polities, the association of birds and more especially those of hymenopterous insects. Such coordination is supposed to have been produced by the operation of natural selection, the almost perfect

* Driesch. Analytische. 'Theorie der organischen Entwicklung.' Leipsig, 1895.
See also Wilson. "The Cell in Development and Inheritance."

ethics of a bee-hive having been attained by the slow elimination of all the independent individuals whose actions were not sufficiently adapted to the welfare of the community as a whole. If we accept the supposition that there is an "ideoplasm," that is to say that during ontogenetic development the cells are sorted out into groups which differ substantively and actively from each other, the histological heterogenesis of an adult multicellular organism may perhaps be conceived as the result, but if we accept Nägeli's idea and couple with it the operation of natural selection, the double conception carries us nowhere in respect to the ordered unfolding of an embryo or the reciprocal working of the adult machinery.

26. By coordination we really mean the control which limits and directs the activities of communities of cells, and the only conception which we can formulate at present is that either through substantive continuity or in some other way the cells of a multicellular organism do influence one another and do act reciprocally. In other words it has been found that the unity of structure and of organization which characterises a single cell can be extended over a multitude of cells.

THE MAIOTIC PHASE.

27. In the life cycle of the protozoa we see that there is a continual alternation between periods of division and acts of conjugation. In the ciliated infusoria the process of conjugation is preceded by a particular form of division of the nucleus into two, followed by another division giving rise to four daughter nuclei. Of these, two degenerate, and of the remaining two one passes into the other conjugating gamete, the process being repeated in each individual.

28. The parental and acquired nuclei now fuse and the process of conjugation is complete. The conjugating forms separate and pass again through the successive divisions which intervene between each conjugational act.

29. There are numerous observations which indicate that in the protozoa the number of chromosomes which appear during each ordinary fission are constant, while there are others which, like those of Hertwig, appear to show that in the last division of the nuclei before conjugation this number is reduced by one half. In this way by the addition of the nucleus passed across from each of the conjugating individuals the chromosome number is brought back again to what it was before this reduction had supervened. The exact manner in which the reduction in the number of the chromosomes is brought about in the protozoa is not at present clearly elucidated, chiefly owing to the extreme minuteness of the nuclei themselves, and partly also because the animals are very difficult to work with.

30. But in the metazoa and the multicellular plants before fertilization a similar reduction in the number of the chromosomes in the cells destined to conjugate has been found to occur, and here the process can be studied with a high degree of accuracy and detail.

31. When, after its completion by fertilization, the egg of a guinea pig begins to segment, and during those divisions by which the original blastomere is split up into a great multitude, the number of chromosomes appearing at each division remains constant, in the guinea pig at 32. Long after the cells which eventually give rise to the different parts of the embryo have been split off, this number still remains constant. Indeed among all the truly somatic structures, the cells to the end of their life, when in division, exhibit the same number of chromosomes.

32. The cells which do not form the true soma, such as the reproductive elements, follow a different course. At the time of the maturation of the sexual glands all the spermatogenetic or ovigenetic cells within them tend sooner or later to pass through forms of division which differ from those that have preceded during their embryological development.

33. These divisions result in the number of the chromosomes being reduced by one half. We have, therefore, in the development of a metazoon, a type of division which characterizes the early segmentation of the egg, and all the subsequent divisions of the blastomeres which go to form the true soma. The same type of division characterizes also the early multiplication of the cells which give rise to the spermatogenetic and ovigenetic elements. Along their track of development, however, the original type of division is succeeded by another immediately before the reproductive cells become ripe for fertilization.

34. We have termed the divisional phenomena which intervene between the early development of the sexual cells and their maturation the maiotic phase, consequently we may speak of the divisions which precede the maiotic change as pre-maiotic divisions, and any which come after it as post-maiotic. In this way all divisions encountered in the soma proper are pre-maiotic, and so also are those encountered in the early development of the sexual cells themselves. The maiotic phenomena occurring in the development of the sexual cells of the metazoa thus correspond to the reduction in the number of the chromosomes occurring before the conjugation in protozoan gametes. The same nomenclature can be employed with regard to the protozoa. We may speak on the divisions which occur among the gametes after conjugation as pre-maiotic, and those taking place immediately before conjugation as maiotic and post-maiotic. In this way it becomes clear that the life-history of the protozoa or unicellular plants is precisely similar to the life-history of the cells of the metazoa and multicellular plants. The development of the protozoa is cyclical. It passes from conjugation to division, from division to conjugation. It is an endless circle of recurring phases. In the metazoa and the multicellular plants, on the other hand, it is only in those cells that do not belong to the somatic machinery that we find this cyclical growth. Starting from the same origin, the fertilized egg, the development of the cells of the soma is not cyclical but translatory, passing through a series of similar pre-maiotic divisions to complete histological differentiation and eventual dissolution.

35. The maiotic phase is found in the reproductive cells of all animals and plants where sexual reproduction is known to occur. It is found also in all those unicellular forms in which conjugational phenomena have been hitherto worked out. It is thus from a biological point of view, a phenomenon of the highest importance, since it appears to be a universal attribute of the sexual reproduction of living things.

36. The exact occurrences that take place in the cells during the maiotic phase have only lately been clearly apprehended, and the whole subject has been greatly obscured by the indiscriminate mixing up of etiological speculation with ascertained facts. The process has, however, now been followed out in a sufficient number of animals and plants to make it quite obvious that its features are invariably similar and to allow its successive phases to be accurately described.

37. We have already seen in the case of *Periplaneta* and *Triton* that the chromosomes can be discerned as individual entities even during the resting stages which intervene between each successive division of the cells. In general the maiotic change is simply brought about

by a pairing of the chromosomes during the resting condition of the nuclei. That is to say, whereas in *Periplaneta* we have 32 chromosomes, during the onset of the maiotic change, these 32 pair into 16 entities or gemini. It is these gemini that have been termed Heterotype chromosomes, but since the term chromosomes applies to a particular thing, it is far better, for the sake of clearness, to speak of the aggregates formed during the onset of the maiotic phase, by some distinct term, such as gemini. At the time the chromosomes pair to form the gemini they become also arranged in characteristic forms within the nuclei. In this way a curious contracted appearance is produced, to which in 1895 we gave the name of synapatic contraction. This appearance is often, but not always, characteristic of the maiotic phase. It does not appear, for example, in the case of *Triton*. In ordinary or pre-maiotic divisions the chromosomes become split longitudinally, and a similar split takes place in each of the two chromosomes that associate to form the individual gemini. In the ensuing first maiotic division, however, this splitting is only incipient and does not result in a separation of the longitudinal halves of the chromosomes of which each of the gemini are made up.

38. In the first maiotic (Heterotype) division it is not the halves of the longitudinally split chromosome which separate, but each united pair of chromosomes disunites upon the spindle, and the entire chromosomes pass intact to opposite poles of the cell. In this way, since in *Periplaneta* there are 32 chromosomes before the maiotic change intervenes, when this happens the chromosomes become united into 16 pairs. These pairs, when upon the spindle, behave as though they were chromosomes, simply disuniting so that 16 pass one way and 16 the other, into the daughter cells. The longitudinal split visible in each constituent of the gemini does not come into effect in the first maiotic division and may be clearly seen in each of the separated chromosomes during the diaster of this division.

39. By the above method of division, since the chromosomes unite in pairs before division, and merely disunite to form the daughter nuclei, it results that each daughter nucleus has only 16 chromosomes, or half as many as there were in the cells before this sort of division supervened. So far as the number of chromosomes is concerned, it is obvious that since the number of these bodies in cells has been found constant, all the cells after the first maiotic division should have only half as many as there were before. This has been found to be the case. In mammals there is only one division after the first maiotic in the history of the reproductive cells. Here the number of the chromosomes remains reduced by one half. But in plants there may be numbers of cell generations after the first maiotic division. In all these the reduced number of the chromosomes is retained.*

* We have recently ascertained the remarkable fact that in the spermatogenises of *Hydrophilus piceus* there a third division following the second maiotic.

PART II.

COMPARATIVE CONSIDERATION OF THE CYTOLOGY OF CANCER.

40. In the present state of knowledge it is not clear, from a cytological point of view, that cancer, as a distinct form of tumour, can be morphologically defined. From a cytological aspect, cancer is an abnormal proliferation of the cells originally forming some portion of an animal's body. Such proliferations, when they are described as cancer, are distinguished from other growths by a number of physiological attributes which it ought, according to existing conceptions, to exhibit. Thus cancer continues to grow and at the same time invades and destroys surrounding tissues. It produces metastases in different parts of the body, and when removed it tends to recur.

41. The definition of what we call cancer is, therefore, constituted by a number of attributes appearing together, and it will be obvious that from the etiological point of view, such a definition is by no means satisfactory. It is clear that growths presenting the above attributes in conjunction might be of the most diverse origin, and etiologically entirely distinct from each other. In practice it is found, moreover, that the attributes which are together regarded as distinctive of cancer, are not always all present, and often not present in the same degree relatively to each other. Some tumours that grow rapidly, invade, destroy, and form metastases, show little tendency to recur. Again, tumours which invade, destroy and recur repeatedly after removal, may not form metastases. Further, tumours which recur and form metastases may be, at least relatively, lacking in invasive and destructive powers. There is, indeed, a general consensus of opinion among surgeons, physicians, and pathologists, that both from the ordinary histological and physiological points of view, it is possible to select tumours that constitute series that pass, almost if not altogether, insensibly from simple hypertrophy to invasion, the formation of metastases, and the most widespread results of destructiveness.

42. It is, moreover, doubtful whether there are any definite microscopical appearances that show that a particular tumour has the full physiological attributes of malignancy. At present it is quite conceivable that there may exist a large class of tumours the members of which are all similar in origin, though exhibiting the attributes of malignancy in different degrees. It is also possible that these attributes may vary in degree from time to time in the same growth. The trend of recent investigations has tended to make it more and more apparent that the problem of cancer is not adequately expressed by the question: "What is Cancer?" but rather by the necessity of ascertaining what has been the precise mode of origin, and cell lineage in particular growths.

43. If we can demonstrate the existence of a class of tumours originating in some particular way from any particular cause, and if these tumours exhibit in individual instances the attributes of malignancy, we shall have solved the problem of the nature of cancer in a particular case, and shall have laid the foundation for the etiology of malignant growths in general.

44. The researches described in the following pages have been conducted with this end in view. When the investigations were begun, the cytology of cancer was practically a blank. It had been shown by Hertwig, Galeotti, von Hansemann, and others, that irregular

mitosis occurred in cancerous growths, and von Hansemann first systematically studied these abnormalities, showing that cells existed in such growths, containing sometimes more, sometimes fewer, chromosomes than the normal complement of the body cells belonging to the animal in which the growths occurred. Von Hansemann also made the further important step of putting into definite language the existing, but indefinite impression that the cells of malignant growths lose, during their development, some or all of their histological differentiation.

45. This phenomenon of "Anaplasia," as von Hansemann called it, together with the abnormalities of mitosis above referred to, constituted the chief cytological characteristics of cancer that had, up to 1903, been definitely recognised.

46. Having ourselves dealt with the phenomena of mitosis in animals and plants generally, our attention was directed to these processes as they occur in cancer and the first part portion of the present report will be concerned with the results of this particular investigation. It will be convenient to arrange the following matter under succesive heads :—

1. Amitosis.
2. Irregular mitosis.
3. Pluripolar divisions.
4. Maiotic and post-maiotic divisions.

I. AMITOSIS.

47. Amitosis is found to occur very frequently in almost all malignant growths. The phenomenon of amitosis, or simple direct division of the nuclei of cells, is of somewhat rare occurrence in the normal somatic tissues of animals and plants, and owing to the fact that it is often in evidence in tissues accessory to reproduction, among leucocytic cells, and in many pathological developments, it has come to be regarded as a phenomenon incidental to the degeneration of the tissues in which it may appear. Our recent observations do not confirm this view. Amitosis occurs regularly in the myeloplaxes of the red bone marrow of mammals and amphibia. It is seen also among the leucocytes found in the marrow, and in neither of these instances is there the slightest reason to regard these cells as on the road to dissolution. There is on the other hand, direct evidence against any such conception. In the bone-marrow amitosis producing several nuclei within a single myeloplax, alternates with mitosis, the nuclei which have been produced all dividing together as in fig. 2. Again, it is found that amitosis occurs regularly among the spermatogonia of amphibia, that is among cells that have been derived through successive mitoses. It is only after these cells have been multiplied by amitosis that they re-assume mitotic activity, and give rise to the spermatogenetic elements of the animal in question. Here then we have a regular alternation of mitosis and amitosis among the male ova of Balfour, followed by mitosis and the production of sexual cells.

48. By these observations it is indicated that amitosis is not necessarily a process related to necrotic phenomena, but that it occurs regularly in alternation with mitosis among a number of diverse normal cells in the bodies of animals. It should also be noticed in this connection that such alternations have only been found as a characteristic of the reproductive elements, and those which like them are out of coordination with the soma. (See pars. 21-26).

49. The amitosis found in malignant tissues is thus matched by similar amitosis occurring normally among the uncoordinate cells of the animal body. It is, therefore, neither evidence of necrosis, nor a special attribute of malignant growths.

2. IRREGULAR MITOSIS.

50. Various authors, von Hansemann, Galeotti, and others, have noted that among the mitoses of cancer there are to be found cells which produce an unequal division of the chromosomes (fig. 3). These elements are somewhat rare, and the exact sequence of events during their division is not very clear. That they are not characteristic of malignant tissue is rendered obvious by the fact that similar irregular mitosis may be produced among normal cells by the action of certain reagents. (See Galeotti, Schottlander and others). Von Hansemann has also drawn attention to the fact that in some of the cells of cancer an irregular breaking up of the chromosomes occurs, and that in such cells some of the chromosomes appear to have been thrown out of the spindle figure. It is through such mitoses that von Hansemann supposes the hyper and hypo-chromatic cells of cancer may originate. We are not clear that hyper and hypo-chromatosis is brought about in the way suggested by von Hansemann, or by the uneven division previously referred to.

3. PLURIPOLAR DIVISIONS.

51. The pluripolar spindle-figures, which are often encountered among the cells of malignant growths, may generally be interpreted as the result of the simultaneous division of more than one nucleus existing in a single cytoplasm. They are precisely similar to the pluripolar spindle figures encountered during the division of the myeloplaxes in normal bone-marrow (fig. 2). It is a curious and significant fact that among the spermatogonia and oogonia in mammals, including man, similar pluripolar figures are encountered with great frequency. In these instances it is easy to follow what happens fairly exactly. In the case of the first maiotic mitosis in man, the division often becomes complete without the cytoplasm dividing. In this way we have two or more nuclei in the same cytoplasm. When such cells pass into the second maiotic division, both nuclei divide together, presenting the appearance of tri- or quadri-polar spindle figures (fig. 4). During such divisions all the chromosomes may become involved upon a common spindle, and only in the later stages of the process sort themselves out into their respective daughter groups.

52. From these observations it is clear that the multinuclear cells and pluripolar divisions found in cancer, are similar to the conditions found normally in the sexual cells and the other elements of the body, which, like leucocytes, are outside the somatic coordination. Like amitosis and the other forms of irregular mitosis we have considered, the pluripolar spindle figures are not characteristic of cancerous tissues.

MAIOTIC PHENOMENA.

53. In 1903 we, in conjunction with Professor Farmer, pointed out that there are occasionally to be found in cancerous growths, division figures that closely simulate the peculiar forms encountered in the sexual glands during the first maiotic (heterotype) division. At the same time we drew attention, in a communication to the Royal Society, to the fact that if these divisions were similar to the first maiotic divisions of the ovary and testis, the

reduction of the number of chromosomes to one half, which is common in cancer cells, would be accounted for. The correct ascertainment of such processes in cancer cells is a matter of extreme difficulty, and the issue of this part of the investigation has been greatly obscured by subsequent publications from other sources. For instance, it has been stated that longitudinal splitting of the chromosomes in certain division figures occurring in malignant growths, proves that these division figures cannot be similar to the first maiotic (heterotype) division occurring in sexual glands.* In reality this longitudinal splitting of the gemini (heterotype chromosomes) is always a normal concommitant of the first maiotic division. This was first observed by Flemming in 1887, and has been universally confirmed by cytologists since that time. So far from being an argument against, it is an additional proof that these figures are similar to the first maiotic division (see pars. 37, 38 and 58).

54. In a subsequent communication to the Royal Society, we referred again to the existence of maiotic phenomena during the growth of cancer, and it is desirable to treat the subject still more fully now.

55. In fig. 5 we have a somatic (pre-maiotic) division figure from the testis of man. In figs. 6, 7 and 8, somatic divisions from human cancer. These are given for the purpose of comparison with the maiotic divisions.

56. In figs. 9 to 17 is a series of drawings of the first maiotic division as it occurs normally in man. In figs. 9 and 10 are represented stages of the synaptic prophase. In fig. 9 it will be seen that the so-called spireme is contracted to one side. In man this synaptic contraction is by no means so well-marked as it is in many other mammals. In the individual stage, represented in the figure, the threads are beaded, but not split. In fig. 10 the thread work has become split, and this early splitting in the synaptic prophase is characteristic of the first maiotic division, for it is not found in the corresponding stages of the somatic division.

57. In fig. 18 we have a stage in the division of a cancer cell which corresponds to the stage represented in fig. 9 from the testis of man. It will be noted that the contraction of the threadwork is not so marked as that represented in fig. 9, but is, nevertheless, quite obvious. Fig. 19 represents a later stage in the division of a cancer cell. It corresponds to fig. 10 and it will be noted that in the cancer cell the thread is split, just as it is in the testicular element. In figs. 11, 12, 13 and 14, we have a later stage in the first maiotic divisions in the testis of man. The gemini (heterotype chromosomes) are formed and their number is half that of the somatic chromosomes. In figs. 20 to 23 similar stages are represented from the cancer series, and here it will be observed that the forms of the gemini in figs. 11 to 14 correspond.

58. In figs. 15 and 16 we have the spindle figure of the first maiotic division in the testis of man. In figs. 23 and 24 the corresponding stage in a cancer cell. It will be noted in both cases that the chromosomes are curiously spread about over the spindle. Fig. 17 represents the appearance of the chromosomes as they separate to form the diaster in man. There we notice the fact first recorded by Flemming, that the daughter elements are longitudinally split (see pars. 37 and 38). In fig. 25 we have a corresponding diaster in cancer, and here again we see that the chromosomes are split in the same characteristic manner.

* Bashford and Murray. 'Proc. R.S.' Vol. 77. No. 13. 516. Page 226.

59. It is thus obvious that in cancers occurring in man, there exist divisions which correspond in all their essential features with the first maiotic mitosis in the same animal. This being so it becomes also obvious when we consider the nature of the maiotic process (see pars. 27 to 39) that in the daughter elements of such divisions there will be only half as many chromosomes as there were before, and it is desirable to consider this matter in relation to cancer in somewhat greater detail. When compared with that of other mammals, the first maiotic division occurring in man is seen to possess characters of its own. The chromosomes as they appear on the spindle have the form of either solid blocks of chromatin, or appear in the various forms given in figs. 11 to 16. It is clear that we are here dealing with the varieties of the synaptic gemini to which we have drawn attention in a recent publication.* It is found in mammals such as rats, mice and the amphibian *Triton*, where the synaptic gemini are relatively easy to examine, that there exist numbers of similar gemini which form groups differing from one another. All these groups, as we have shown, are obviously built up in the same way. That is to say, whatever form the gemini may assume, they are formed of two chromosomes united or associated together. In the case of the first maiotic division in man, the gemini are often so contracted and solid that it is not easy to say how many forms actually exist, but it is perfectly clear that there are at least six types of such gemini (see page 76). In the heterotype divisions occurring in human cancer, the number of forms assumed by the gemini is apparently less, and we have only been able to distinguish clearly the forms given in figs. 20 to 24. Thus it would appear that the first maiotic division in cancer differs from the normal first maiotic division in man in the forms of its gemini, in much the same way as the first maiotic divisions in rats and mice differ from one another.

60. As we have already suggested, the formation of the different groups of gemini in the first maiotic divisions of different animals possibly depends upon specific differences in the parental chromosomes which unite to form the specific gemini. In cancer, considering the phenomena encountered in connection with leucocytes (see pars. 71 to 75), that there should exist variations in the gemini is at least what would have been naturally anticipated.

61. In the existence of the maiotic form of division in human cancer, we have then one more instance of the type of mitosis corresponding with the normal mitosis encountered in the reproductive cells and the other non-coordinated elements such as leucocytes.†

62. On consideration of the foregoing observations, it will be seen that none of the varieties of division encountered in cancer are specific of malignant growths. They are all matched by varieties of mitosis occurring normally in the reproductive and other uncoordinate elements of the animal body. The varieties of divisional phenomena run parallel with what takes place normally in the sexual and uncoordinate cells. The existence of heterotypical mitosis in cancer is however much more striking than the rest. Maiotic phenomena are peculiarly characteristic of sexually reproductive cells, and the fact that this kind of mitosis exists in cancer shows conclusively that there must exist some community of nature between cancer and reproductive tissue.

63. It has long been known that together with the nuclear changes appearing during the maiotic phase, other characteristic changes take place in the cells involved. Thus, as

* Moore and Arnold. "On the existence of Permanent Forms among the chromosomes of the first maiotic division in certain Animals." Proc. R.S., 1906. *See Appendix.*

† C. E. Walker, *loc. cit.*

Meves and others have pointed out, the archoplasm becomes more prominent during synapsis before the first maiotic division. This structure is prominent in each of the generations which, in vertebrates, succeeds the first maiotic division. In 1895 we drew attention to the fact that in mammals during the synaptic prophase, the centrosomes, which during the preceding generations occupy the middle of the attraction sphere or archoplasm, wander out and are found outside it in the cytoplasm. About the same time the archoplasm becomes filled with minute clear spheres. These, together with the archoplasmic substance, vanish in the later phases of the first maiotic division. In the daughter elements, the same phenomena are repeated. After the second maiotic division, we find the same relative displacement of the sphere and centrosomes. In the spermatid, the centrosomes remain outside the archoplasm, and the latter body develops vesicles in its interior. In the spermatids these vesicles proceed further than in the preceding maiotic generations, and one of them, developing to a large size, is finally converted into the cephatic cap of the spermatozoon. When fully developed (fig. 26) the archoplasmic vesicle consists of a distinct membrane containing a clear fluid with a central darkly-staining mass. The formation of archoplasms and vesicles is not peculiar to man or to mammals. They are found during the maiotic period in many vertebrates, even in the Elasmobranch fishes, and are similarly encountered among a wide range of invertebrates, including worms, Arthropods, and Mollusca. The importance of these bodies lies in the fact that they form a peculiar and characteristic feature in the cells which are undergoing or have undergone the maiotic change.

64. In 1903, Borel drew attention to the fact that some of the so-called cancer bodies were similar in appearance to the archoplasm and its vesicles in the spermatids of the cobra. He regarded these bodies as hypertrophied centrosomes. That this conclusion could not be maintained was shown by Benda and others, who pointed out that centrosomes coexist in the cancer cell with the vesicles.

65. In a paper published in the Proceedings of the Royal Society, 1905, we drew attention to the fact that the type of cancer bodies, commonly known as "Plimmer's bodies" are strictly comparable in mode of formation and character to the normal archoplasmic vesicle in spermatic cells. We pointed out further that where Plimmer's bodies are found in cancer, the same curious displacements of the centrosomes obtains as in normal maiotic cells. We have then in the existence of Plimmer's bodies in cancer no suggestion of any parasite, but another striking indication of the presence of maiotic phenomena in the cycle of malignant growths.

66. It is perhaps desirable to point out here that great discrimination and care are necessary in recognising the true "Plimmer's bodies" in cancer. As in reproductive glands, so in cancer, a large number of cells degenerate, resulting in the presence of numerous nuclei which appear as shown in fig. 27.

67. Such degeneration figures will be familiar to everyone acquainted with the histology of cancer, and they are to be carefully distinguished from the real archoplasmic vesicle, with which they have no affinity. Again, as is well known, cancer cells often engulf other cells of various sorts, and the latter elements, in degenerating within the cancer cells may produce appearances closely simulating the true archoplasmic vesicle (fig. 28).

PART III.

DEDUCTIONS CONCERNING THE ETIOLOGY OF CANCER.

68. The foregoing considerations will have made it obvious that during the growth of cancer cytological phenomena are exhibited, the characters of which are closely parallel with those encountered in the formation of normal sexual elements. In dealing with the etiology of cancer, this fact which may now be considered as clearly established in relation to a number of typically malignant growths, will have to be considered and taken into account.

69. When we first encountered the above phenomena, it seemed possible that a large proportion of the cells constituting malignant tissue might consist of elements that had passed over to the maiotic phase, much in the same way that the prothallial growth of ferns consist usually entirely of reduced cells. In view of this we have had a series of countings made of the number of chromosomes appearing in the cell of a number of typically malignant growths, our object being to find the number of chromosomes present in cancer cells, and the relative proportions of those cells with the somatic number of chromosomes, those with more and those with less. Inspection of the diagrams given on pages 45 and 46 will show that the relative proportions of reduced and unreduced cells varies considerably in different growths. Thus we may have a growth possessing a considerable number of cells exhibiting the normal somatic number of chromosomes, along with these, others exhibiting gemini in about half this number, and yet other cells that possess the reduced number of chromosomes, but are dividing in the pre-maiotic manner. This last group probably corresponds to the post-maiotic cells in plants. Further, there are cells that exhibit chromosomes in numbers lying between these normal ratios. On reference to page 46, it will be seen that there is a maximum of 24, which, though small, is quite distinct. On comparing the above curves with curves produced by counting the cells in a normal sexual gland in the same way, we find that we have here the same two chief maxima, one at the normal somatic, the other at the reduced or maiotic number. In the normal sexual gland, however, there is no indication of the intermediate maximum 24, which is shown in the cancer curves. It may be said, then, that in a typical malignant growth, there exists an association of pre-maiotic and maiotic nuclei in varying proportions, just as they vary in a similar way in the functional sexual gland. The cancer tissue differs, however, from the sexual gland in possessing cells with chromosomes ranging closely round another maximum. The foregoing observations establish two things clearly. First, an extension of the parallel between cancer and reproductive tissues. Secondly, the result of counting the chromosomes in cancer cells has been to show that these growths do not consist entirely of reduced cells like a normal plant prothallus, nor yet of cells with the normal pre-maiotic number of chromosomes, but of cells the nuclei of which contain chromosomes in reduced, unreduced, and certain other numbers in varying proportions.

70. If examples of malignant growths be selected at random, and their cytology examined, it will be found that they differ widely in the case with which maiotic phenomena of any particular kind are to be found. In young growths, reduced cells and cells exhibiting the first maiotic mitosis are relatively common. Yet even in the same growths the relative proportion of the cells which exhibit these phases may vary very greatly indeed. The inference from these facts seems to be that the maiotic phenomena in cancer cells occurs

periodically, and alternates with other periods of activity. In metastases, and in old primary developments the cells exhibiting the first maiotic division are few and far between. Reduced cells dividing in the pre-maiotic manner, and consequently to be regarded as post-maiotic, are, however, common. So also are cells with larger numbers of chromosomes which divide in the pre-maiotic manner, and are consequently to be regarded as corresponding to the similar cells in the reproductive glands and in the soma. From the above observations it appears to be indicated that in growths of long standing there arise successive crops of maiotic divisions and their progeny, but along with these and giving rise to them there exists a numerous class of cells dividing pre-maiotically.

71. In very young growths the cytological characters are very different, and as a means of elucidating the above conditions, deserve particular attention. Fig. 29 shows a general view of a young primary epithelioma of the lip. On the upper and right hand side of the figure is seen the normal epidermis, which, as we pass to the left is hypertrophying and becoming gradually converted into the anaplastic and typically malignant growth that is visible in the lower portion of the same figure to the left. In the normal portion of the epidermis it will be noticed that the Malpighian layer contains a few leucocytes lying embedded among the prickle cells. There are not many of these bodies, and their activity and number seem to be about the same as in perfectly normal epithelium. As we pass from the region A to B it is seen that the Malpighian layer is thickened slightly, the prickles between the individual cells have become longer, and there is a relatively enormous increase in the number of the leucocytes that have been attracted to the area, and burrowed between the epidermal cells (not fully shown in the figure). Between the regions A and B, some change has come over the epithelium, accompanied by a slight increase in its bulk, and an enormous increase in the attraction it exerts upon the wandering cells.

72. An epithelium may be stimulated to attract leucocytes in a variety of ways. Thus if it is rubbed, or scarified, or burned, or injected with turpentine, a similar concourse of leucocytes to the stimulated area takes place. After the inflammation produced subsides, the leucocytes disperse, and the epithelium returns to its normal condition. In the case of the production of cancerous tissue the process involving the attraction of the leucocytes goes much further than this. As we pass from B to C, we find that at the same time as the epidermis is becoming distended and filled with leucocytes the cells of the Malpighian layer are proliferating, and forming long down-growths, into which the eruction of leucocytes continues unabated. In such a region we also constantly find cells like those represented in figs. 30 to 34, where a tissue cell contains within its cytoplasm a leucocyte or more than one leucocyte. It has long been known that leucocytes were sometimes to be found in the cells forming malignant growths, and it has generally been assumed that this fact indicates that the cells of malignant growths have become phagocytic with respect to the leucocytes, or *vice versa*. Closer investigation of this matter has, however, revealed the fact that neither of these views can be regarded as satisfactory. We have frequently encountered cells in young malignant growths in which there existed one or more leucocytes, whilst either the nuclei of the cells, of the leucocyte, or of both were in a state of active division (figs. 35 to 38). In these cases the spindle figures may be independent, or they may become mixed. In the latter case it is clear that there must be an intimate blending of the substance of the leucocyte nucleus, and that of the cancer cell, and in any future generations that may arise from such cells these cannot be regarded as either somatic or leucocytic in

nature, but are, in the strictest sense of the term, a hybrid product of both. In a certain number of cases we have found that after the intrusion of the leucocyte, there is a gradual approximation of the two nuclei without any division until they become closely applied to one another (figs. 39 and 40). Here the membranes of both nuclei remain distinct, but in others there is a tendency to the formation of a common capsule which encloses both nuclei. Later stages of this process are not difficult to find, and, as will be seen in fig. 41, all trace of the membrane separating the nuclei may have disappeared, although the "polymorphic" chromatin masses are still visible in the conjoint nucleus.

73. It is thus indicated that in cancer formations there exists a curious blending of leucocytes and tissue cells, which does not result necessarily in the destruction of either participant in the association. In consequence of this, there arises a hybrid tissue which is a new formation.

74. The phenomena we have just described have been encountered in a considerable number of individual growths. We have seen them in several examples of carcinoma of the lip, in rectal carcinomata, in epithelioma of penis, and scirrhus of breast. In all these cases the fusions could be definitely related to a particular phase in the genesis of the tumour, the tumours themselves being all very young examples of cancer. From the relation of the fusions to the growing edge, it is obvious that the process extends over a considerable time, and it may actually be continued over the outer edge long after the older portions of the growth have acquired considerable dimensions. Indeed we do not see that there is any limit to the continuation of the fusions. In the older portions of the growth, however, fusions are rarely encountered, nor are they, so far as we have been able to ascertain, encountered in metastatic developments. The matters dealt with in the last section cannot, however, be said to have been driven as yet to a conclusion.

75. It has been found that, during the development of cancer, remarkable interactions take place between the tissue cells that are becoming cancerous, and the attracted leucocytes. These interactions could be interpreted along the lines originally suggested by Klebs, and regarded as a process analogous to fertilization. As we have seen, when they are viewed in this light they certainly tend to explain many of the outstanding difficulties with regard to cancer, such as the anaplasy of malignant tissues and the chromosome numbers peculiar to cancer cells. Yet from the observations it is not clear that this process is really analogous to fertilization. The fusions, on the other hand, might be regarded as some abnormal phagocytic action, and as having no real part in the production of gametoid tissue. The matter requires further investigation, and all the more so because the fusions with which we have been concerned are either simply an incident of the onset of cancer, or they are themselves the direct cause of the formation of gametoid tissue.

76. What it is legitimate to conclude at present may be briefly stated as follows:— There are tumours possessing the attributes of malignancy, and which at the same time are gametoid in structure. So far as is known, gametoid tissue is not encountered in tumours that are not malignant, but it would be rash to say at present that all gametoid tumours are malignant. Gametoid tissue is so complex and peculiar that from a morphological point of view it seems almost impossible to escape the conception that all tumours with these characteristics may be similar in origin. In other words, that gametoid tumours form a natural class of pathological growths, which are similar in their cytological morphology and manner

of development. Further it has become clear that these tumours are either directly caused by the fusions previously discussed, or the fusions are incidental in the development of gametoid tissue, which starts in response to some other stimulus.

77. In either case our original suggestion that the active part of cancer may be regarded as the equivalent of prothallial tissue in plants, remains intact. In relation to this matter it may be observed that there is no weight in the criticism that the cells of cancer generally possess the somatic complement of chromosomes. If this assertion were true, it would not imply that the active portion of cancerous tissue is dissimilar to a prothallial growth, for it has been shown that the cells of a prothallus may exhibit the full somatic number of chromosomes. It is, however, hardly worth while discussing this matter at length, since the assertions made regarding it are without foundation in fact. In all gametoid tumours examined by us the mass of the cells do not exhibit the somatic number of chromosomes, on the contrary, as von Hansemann originally pointed out, they exhibit an association of cells with more than the normal, with less than the normal, and with the normal, number of chromosomes. The relative proportions of these elements varies in different parts of the same tumour as well as at different periods of its growth. The relative proportions of the numbers of chromosomes found in the cells of cancer may be readily seen in the curve shown on page 46, which represents the results obtained by counting the chromosomes in 400 cells chosen indiscriminately. A comparison between this curve and that obtained in a similar manner from a normal sexual gland (page 46), will make any further comment upon the statement that cancerous tissue is not gametoid in nature quite unnecessary. It is advisable, however, to refer briefly to certain statements published with regard to the results obtained from the transmissible tumour in mice, portions of which were placed at the disposal of several laboratories by Professor Jensen. It has been stated that in the grafts from this tumour, the division figures encountered show the "full somatic number" of chromosomes, which is given as 32. To begin with, it is an ascertained fact that the somatic number of chromosomes in the mouse is 24, and not 32. Secondly, several pathologists have explicitly doubted whether these growths can justly be considered as cancer at all.*

78. The problems which now lie immediately before us are, in the first place, which of the two conceptions referred to is correct. In the second, to ascertain what it may be which either initiates the fusions or determines the transmutation from somatic into gametoid tissue.

79. As we have already stated elsewhere, neither conception precludes the possibility that the initial alteration may be in the nature of a response on the part of the soma to the physical or chemical activities of some parasite. There seems, however, to be some difficulty in supposing that a parasite can have anything more than a transitory connection with the formation of a gametoid tumour. For instance, it has been found repeatedly that the cells of cancer are destroyed by grinding them up, and that the material thus produced will not initiate new growths when introduced into the bodies of the same animal. If there is a parasite permanently associated with cancer it must be of very minute size, and it seems, perhaps, unreasonable to suppose that the mere mechanical destruction of the cells in which it lives could prevent its exerting its characteristic action upon those with which it has been brought into fresh contact.

* I. Michaelis, "Uber den Krebs der Mäuse." J. Erdheim, "Zur Morphologie der Maus-geschwülste," both in Zeitschrift für Krebsforschung. Berlin, 1906.

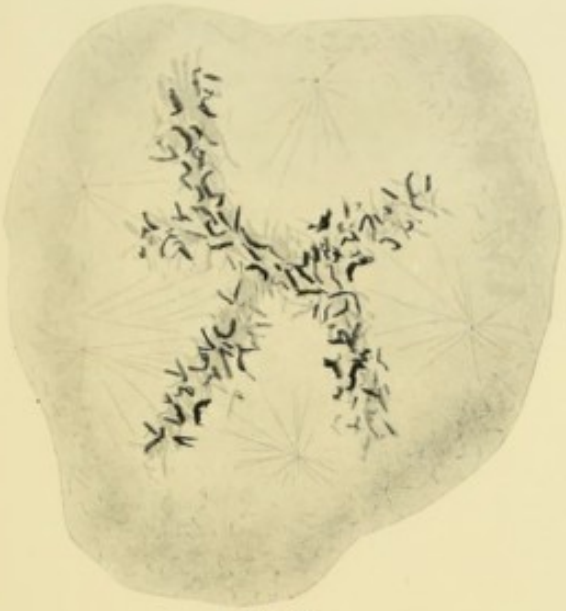


Fig. 2

Fig. 3

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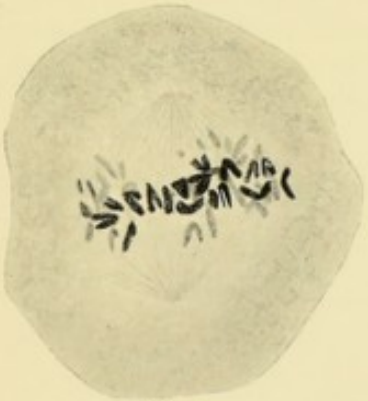

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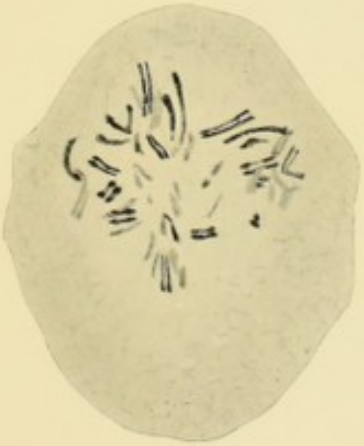
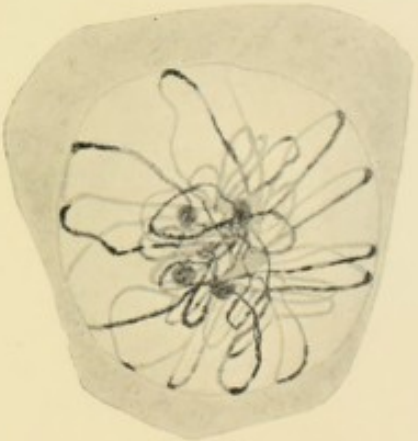


Fig. 8



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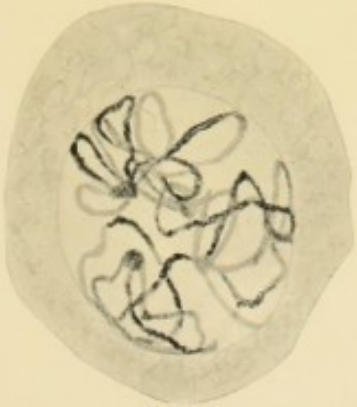

Fig. 10

Fig. 11

Fig. 12

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Fig. 13

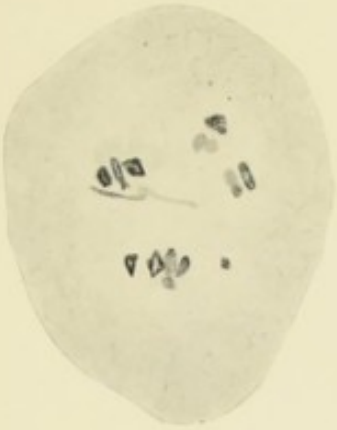

Fig. 14

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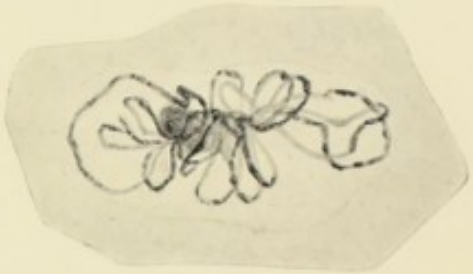

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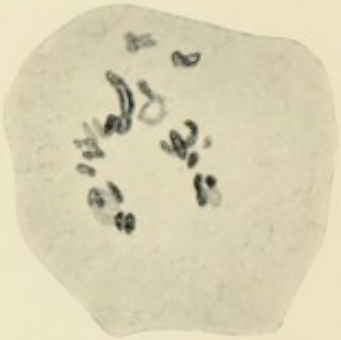
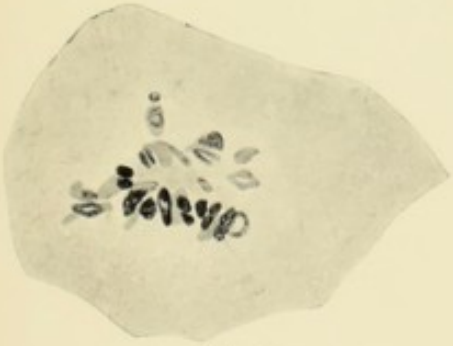


Fig. 21



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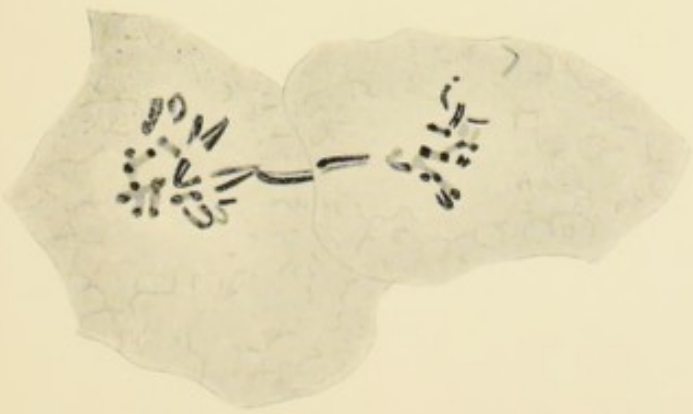

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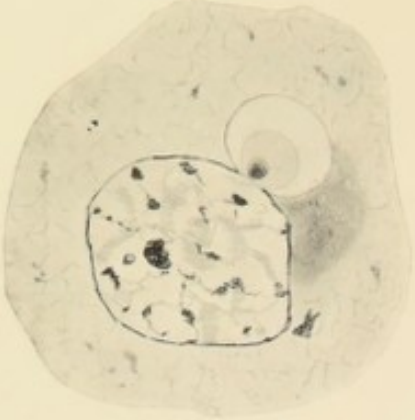
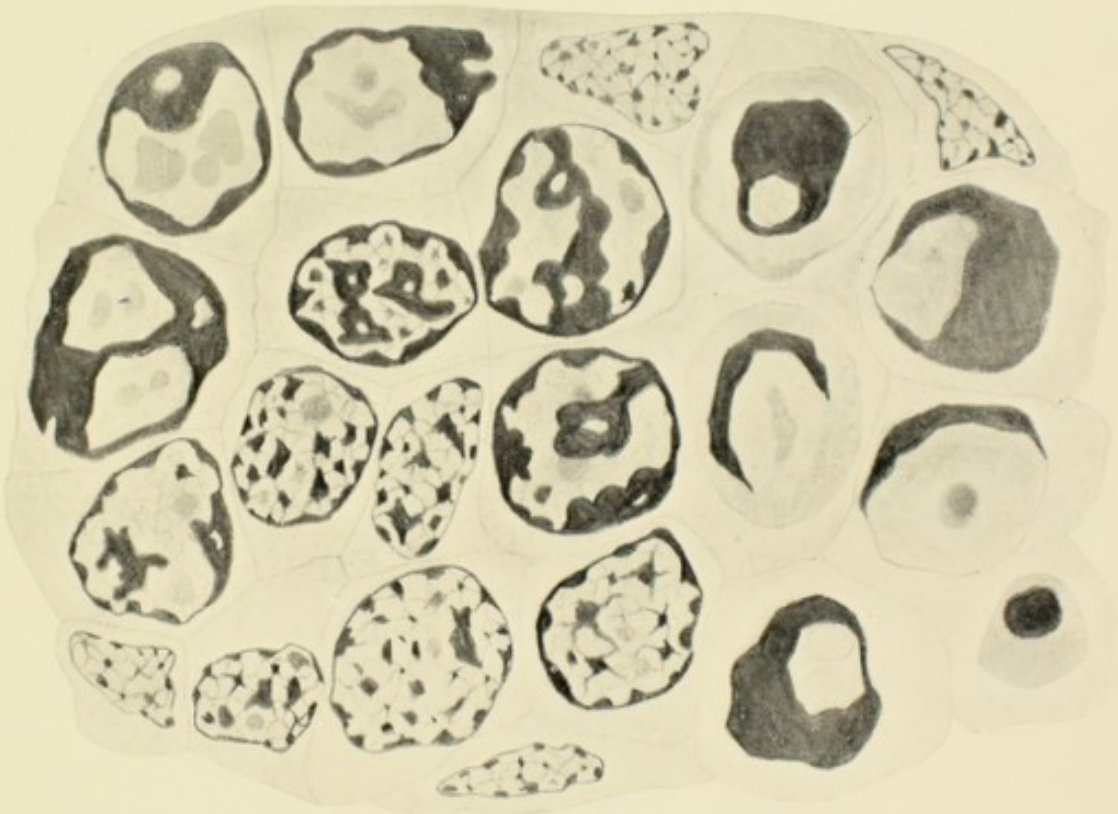


Fig. 26



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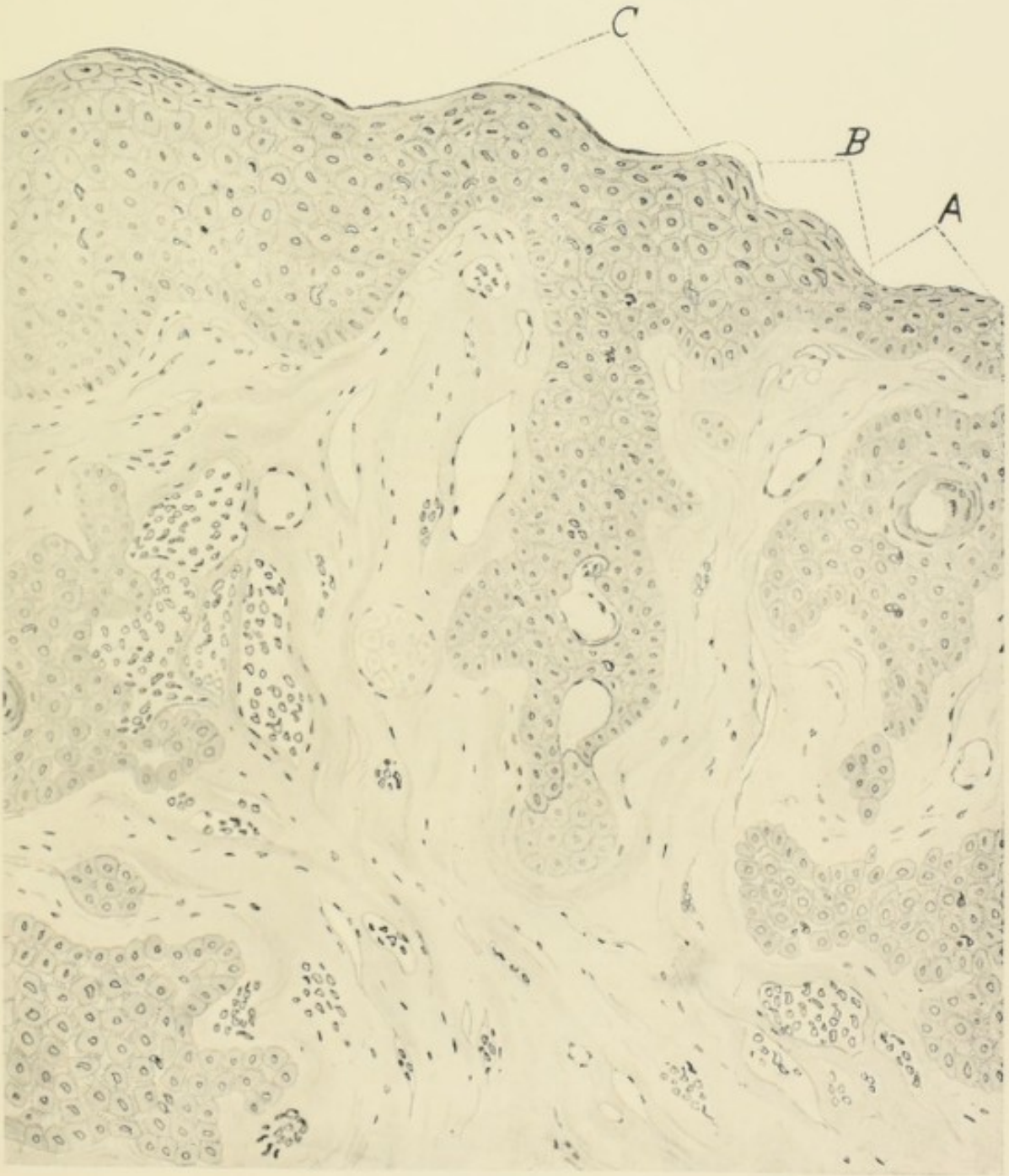

Fig. 29

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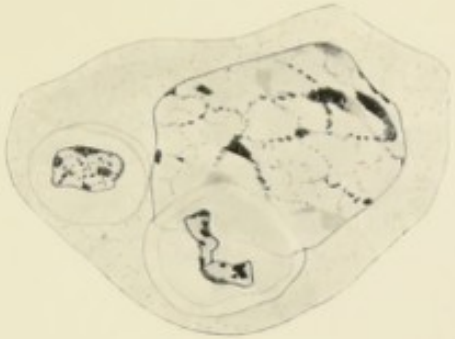

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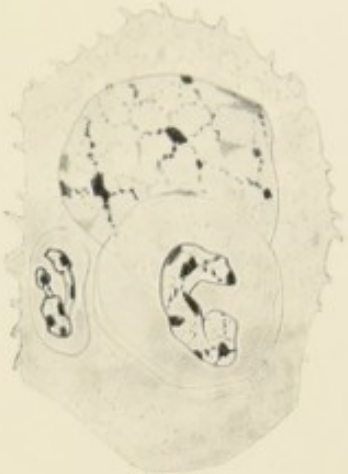
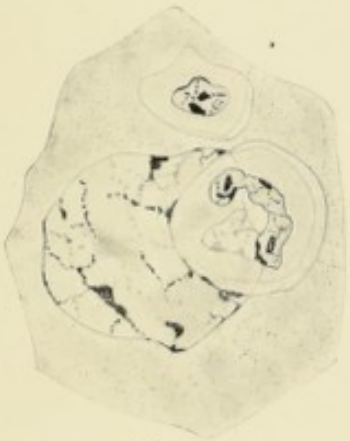


Fig. 32



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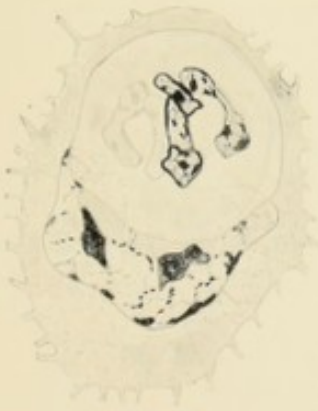

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Fig. 40

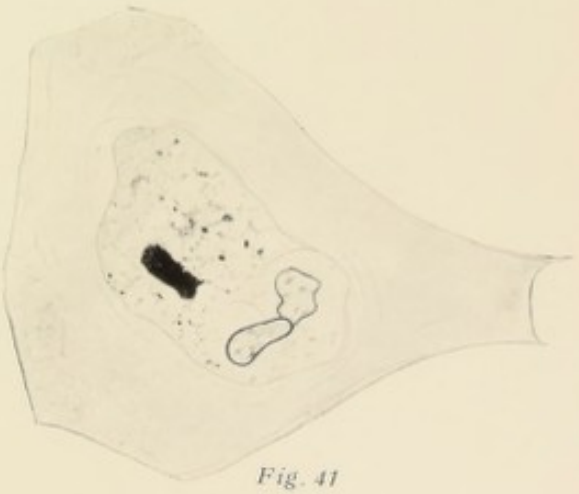


Fig. 41

APPENDICES.

1—ON THE RESEMBLANCES EXHIBITED BETWEEN THE CELLS OF MALIGNANT GROWTHS IN MAN AND THOSE OF NORMAL REPRODUCTIVE TISSUES.*

By J. Bretland Farmer, F.R.S., J. E. S. Moore, F.L.S., and C. E. Walker.

Received December 8,—Read December 10, 1903.

[From the Proceedings of the Royal Society, vol. 72.]

The object of this communication is to draw attention to certain important cytological transformations exhibited during the development of malignant growths in man. We believe that the changes we are about to describe are diagnostic of malignant as opposed to those of a benign character. Furthermore, if our conclusions are well founded, they may at the same time serve to throw light upon the nature of the processes involved in the formation of these growths, and we hope that they may also serve as a point of departure for further investigations on the remote etiology of the disease itself.

We wish, however, at the outset, to disclaim all intentions of formulating at the present time any theory as to the nature of these various remote causes, although, as will be seen in the sequel, our observations indicate certain directions along which such causes may perhaps be profitably sought.

We may at once state as the results of our investigations on a large number of malignant growths, including numerous examples of *Carcinomata* and *Sarcomata*, that we have been able to trace in detail a number of definite and serial changes in the cells of the invading and proliferating malignant tissue, which are remarkably similar to those obtaining during the maturation of the elements contained within the sexual reproductive glands, and it would seem that such a resemblance, extending as it does to minute points of detail, can hardly be destitute of grave significance.

In order, however, to make the position clear, it will be necessary briefly to consider the essential features in which the gametogenic† tissues which are destined to become the reproductive elements are found to differ from the other elements or cells of which the body or soma of an animal or plant is elsewhere composed.

* We desire to state that whilst working together at this subject we have each approached the problems from an independent standpoint. The paper is in every sense a joint one.

† The term *gametogenic*, as here proposed, is also intended to include the primary sporogenous tissue of plants; in spite of possible objections that may be raised, we have decided on this course to secure consistency of expression. [*Note added December 16, 1903.*]

When the egg of an animal or a plant segments to give rise to an organism, the nuclei of all the resulting cells are found to contain a definite number of chromosomes during each nuclear division. These chromosomes pass through a constant series of evolutionary changes. At first the material out of which they originate appears as an aggregation of granules of a stainable substance (chromatin) which finally gives rise to definite structures, the chromosomes. These latter are constant in number for each species of animal or plant, and each of them divides longitudinally into two daughter-chromosomes.

The chromosomes at this period of division become arranged in a very definite manner on the spindle, frequently appearing as V's with the apex directed towards the axis of the spindle. The daughter-nuclei are formed by the distribution to either pole of the respective halves of each original chromosome, and the nuclei thus formed may then enter on a condition of complete rest. Whenever new somatic cells are formed in the body the nuclei pass through identically similar phases. But in every individual there are certain gametogenic cells that are destined to give rise, not to the specialised tissues, but to the sexual reproductive elements. Such cells may be differentiated at a very early period in the embryonic ontogeny of the organism, or they may only become recognisable at a later stage. At whatever stage they may be formed, however, their further history is entirely different from that of the surrounding somatic tissues. The difference in question first becomes apparent as the cell commences its preparation for division, and it is distinguished both by its highly peculiar appearance, and by the fact that at a definite stage in the multiplication of the cells of this reproductive tissue, each unit that is about to give rise to actual reproductive cells passes through a series of metamorphoses wholly dissimilar from those of the surrounding tissues as well as of those of the antecedent cells by the division of which such a cell has sprung. To the peculiar form of mitosis associated with the metamorphosis the term *heterotype* has been applied, and it is a characteristic and interpolated stage in the reproductive cycle of all sexually propagating higher animals and plants.

The essential features wherein this heterotype mitosis differs from those of the body or soma of the organism (whether plant or animal), as well as those in the cell-generations of the reproductive tissues that have preceded it, are as follows:—

1. The period of rest and growth.

2. The chromosomes where they are formed from the resting nucleus are present in only *half the number* of those occurring in the rest of the dividing nuclei of the organism.

3. The forms exhibited by these chromosomes are strikingly different from those of other nuclei. They produce figures resembling loops, rings, aggregations of four heads, and so on.

4. Their division on the spindle is transverse and not longitudinal.

It will be thus seen that this heterotype mitosis is an easily recognised phase in the history of the development of the sexual cells, and for our purpose this is the essential point. With its theoretical interpretation we are not here concerned.

But it is a fact of the highest importance that when once the heterotype division has supervened, all the descendants of that cell retain the reduced number of chromosomes in normal cases. The cycle of these cell-generations, the nuclei of which only form half the somatic number of chromosomes, normally closes with the formation of the definite sexual cells. It is on the fusion of two of these (ovum and spermatozoon) that the double or

somatic number is restored, and this number is characteristic of the fertilised egg, and of all the cells to which it gives rise, until the heterotype mitosis again supervenes in the reproductive tissues. Now after the intervention of the heterotype division, the cell in which it has occurred may, after one further division, at once give birth to the four sexual cells, as in the higher animals, or, on the other hand a varying number of cell-generations may be intercalated before the final differentiation of the sexual elements. This occurs in the majority of plants. It is in these latter that the commonly parasitic character of the organism thus arising is specially, though not exclusively, apparent. Thus, the embryo sac of many flowering plants exerts a destructive influence on the cells of the soma adjacent to it. This property is not, however, by any means exclusively confined to the post-heterotype formation (the gametophyte of the plant), and we do not wish to lay distinctive weight upon it. In the lower plants the bulk of the body is composed of cells with reduced nuclei, and the alternate stage in the life circle, originating in the fertilised egg, is the predatory structure. What seems to emerge from a general consideration of the whole range of facts is this: that in the higher animals and plants the post-heterotype tissue, with its own independence of organisation, does behave towards the surrounding tissues of the parental individual as a neoplasm. So far as the parent is concerned, the new growth might be described as a pathological one, did it not form a normal stage of the life history of the species.

We have said that the cells from which the heterotypically dividing elements will finally arise can often be distinguished from those cells which will not produce such elements. In the testis of a mammal or in the sporogenous tissue of a stamen we recognise with ease and certainty the existence of these cells. They continue to multiply, and though differing from the adjacent cells in many respects, they continue to resemble them in their mode of nuclear division until they pass severally into the peculiar state of growth that ushers in the heterotype division.

In our studies of abnormal growths occurring on ferns, we were struck by certain features presented by the proliferating tissues that are formed during apogamy and apospory, and we have thus been led to make a systematic investigation of the cytological features presented by malignant growths in man.

This has resulted in the recognition of the existence of a surprising degree of similarity between the phases that characteristically recur in such tissues and those transformations of somatic cells into reproductive tissues in general.

Thus in a typical example of rapidly growing epithelioma it is seen that in the early stages of the proliferation of the Malpighian layer, the cells of the invading tissue at first pass through a cycle of somatic divisions, exactly as in the early stages of reproductive tissue. The resemblance may extend to the frequent production of giant cells, a common occurrence in each case.

As cell multiplication proceeds, however, a change passes over the cells themselves. The protoplasmic continuity, to which the "prickly" character is due, becomes more or less obliterated, and the cells assume that appearance of indifferent germ tissue so well known as a feature of the elements of which malignant growths are largely made up. But, in addition to this, other important changes occur which seem to have been generally overlooked.

A varying number of cells, situated in a zone behind the growing edge of the advancing neoplasm, may be observed to attain somewhat large dimensions. Each contains a nucleus

that grows to a considerable size. As the latter enters on a prophase of division, it is recognised that the chromosomes, instead of appearing as delicate thin rods or V's, which are split longitudinally, present the appearance of short thickened loops or rings, closely resembling the later prophase stages of the heterotype mitosis in the normal reproductive tissues. What is still more significant is the fact that in these cells the number of the chromosomes is obviously *less than* in the *normal somatic cells of the surrounding tissues*. In many cases we determined the numbers to be approximately halved as compared with those of the latter. Furthermore, it is clear that the loops and rings characteristic of this stage of the cellular development of the malignant growth are arranged lengthwise on the spindle, and so are ultimately divided transversely, exactly as in the corresponding heterotype mitosis of the reproductive elements.

Subsequent divisions that occur behind this zone appear to resemble the somatic form, but retain a reduced number of chromosomes, just as do the cells that arise from a parent cell that has once exhibited the heterotype character. But irregularities of various kinds usually supervene—amitosis is of frequent occurrence, and the number of the chromosomes in those nuclei that may continue to divide mitotically often exhibits irregularity. These facts do not, however, seriously affect our position, for in many plants similar irregularities occur in post-heterotype cells that are not destined to give rise to actual sexual cells.

The above-described series of cellular and nuclear changes are not confined to epitheliomata, but recur in an essentially similar manner in other carinomata and sarcomata. For example, in a rapidly developing growth of a sarcomatous type from the *cervix uteri* we were able to distinguish near the growing edge a well-marked zone of cells, characterised by the somatic (and amitotic?) types of mitosis, whilst this was succeeded towards the interior by a band of heterotypically dividing cells, and within this again the cells showed the somatic type with reduced number of chromosomes, together with other cells, in which a mitosis was going on.

In the case of slow-growing tumours which obviously tend to produce a considerable amount of normal somatic tissue, such as the fibrous tissue in scirrhus of the breast, cells showing these phases are, as would naturally be expected, far more difficult to find than in rapidly growing tumours. In such growths, cells showing the figures of ordinary somatic division are numerous in comparison with those showing heterotype figures. This would seem to indicate that the cells which are destined to form fibrous tissue never divide heterotypically.

It thus becomes evident that in a most important respect the various types of malignant growths present certain features which are common to all, and that these features are similar to those to be observed in the process of differentiation of reproductive cells from the preceding somatic tissue. We feel that the evidence justifies us in deliberately correlating the appearance of these "gametoid" neoplasms with the result of a stimulus which has changed the normal somatic course of cell development into that characteristic of reproductive (not embryonic) tissue.

We look, then, upon this transformation as representing the immediate cause of the development of the malignant growth but the remote cause must be sought for amongst those various stimuli, some of which, *e.g.*, continuous irritation, are known to favour their development.

Malignant growths seem, furthermore, to be definitely separable from benign tumours, inasmuch as in the latter we have never succeeded in discovering anything resembling the very characteristic nuclear changes we have described above. Thus, *inter alia*, while we have in the example of a polypoid papilloma observed a considerable number of somatic mitoses with the full (unreduced) number of chromosomes, we have been wholly unable to find a single instance of a heterotype division, or anything indicating that a reduction in the number of chromosomes had taken place.

In this preliminary communication we do not propose to deal, except in the most brief manner, with such questions as the probable transmission of the disease from one individual to another, or to its prevalence in certain districts, or even in certain houses.

It seems probable that actual contact does in some cases transmit the disease, but it is apparently equally probable that this happens where cells from the growth are transplanted to another part of the same individual, or to another individual under very peculiar conditions, which allow the repeated application of a suitable stimulus, or of the continuous introduction of cells which have undergone the changes we have described.

In the case of localities where malignant growths are apparently prevalent, *e.g.*, cancer houses, the phenomenon is directly comparable to the occurrence of abnormal cellular developments under suitable stimuli to which we have already referred.

In conclusion we would point out that the various changes which we have described as occurring in cells are always rapid, and possibly hastened during the approaching death of the tissue. Unless, therefore, the tissues are treated in such a manner as to fix the cells composing them some time before death supervenes, the nuclei will be found either in a condition of rest or in one of more or less disintegration. We have emphasised this fact because, in the ordinary pathological methods of preparing specimens, it has not hitherto been found necessary to make proper provision for the preservation and fixation of the cells, either with regard to time or suitable reagents. Such preparations, though, of course, admirably suited for ordinary histological investigation, are not suitable for elucidation of the finer cytological characters of the individual cells.

We cannot bring this communication to a close without expressing our great indebtedness to Dr. W. R. Dakin and to Messrs. Allingham, Baldwin, English, Jaffery, Parsons, Sheild, and others, who, by so kindly enabling us to obtain the necessary material, have made this investigation possible.

2—ON THE RESEMBLANCES EXISTING BETWEEN THE "PLIMMER'S BODIES" OF MALIGNANT GROWTHS, AND CERTAIN NORMAL CONSTITUENTS OF REPRODUCTIVE CELLS OF ANIMALS.

By J. Bretland Farmer, F.R.S., J. E. S. Moore, and C. E. Walker.

(Received April 11—Read May 11, 1905.)

[From the Proceedings of the Royal Society, B, Vol. 76, 1905].

It is proposed in the present communication to present the results of investigations bearing on the nature of those remarkable structures known as "Plimmer's Bodies."* As is well known, these are found in many cancerous growths, and are most commonly encountered in the younger, or growing regions of the tumour. They appear in the form of vesicles, and they consist essentially of a fairly well-defined wall containing a clear space in which is suspended a small darkly staining granule (fig. 1). They are most commonly to be met with in tumours of a glandular or glandular-epithelial origin.† They lie in the cytoplasm of the cancer cell, and usually in close proximity to the nucleus. In size, they vary from excessive minuteness to that of the nucleus itself.

The special interest attaching to them depends on the fact that they have commonly been regarded as peculiar to cancerous cells, although Honda‡ believes he has occasionally also encountered them in inflammatory tissues. They have been variously interpreted. Some investigators have regarded them as parasitic organisms, more or less intimately connected with the etiology of the disease, whilst others have seen in them a differentiation of the cytoplasm of the cancerous cell itself. It has been suggested also that they might be derived from the centrosomes within the archoplasm,§ but the observations of Benda‖ that centrosomes co-existed independently of them in the cell, has rightly been held to disprove this hypothesis.

* Plimmer, 'Practitioner,' vol. 62.

† Greenough, '3rd Rep. Caroline Brewer Croft Cancer Com.,' Harvard Med. School, 1905.

‡ Honda, 'Virchow's Archiv,' vol. 174.

§ Borrel, 'An. Inst. Past.,' vol 15. This author was on the right track in attributing importance to the archoplasm, but the erroneous interpretation placed on the centrosomes precluded his arriving at a satisfactory conclusion as to the nature of the bodies under discussion.

‖ Benda, 'Verh. deutch. Gesellsch. f. Chir.,' 1902.

Our own investigations indicate, however, that there are good grounds for reconsidering the whole position, and a comparison of the processes that normally obtain during the final stages of development of the reproductive elements in man and the other mammalia, appear to us strongly to suggest that a parallel between the "Plimmer Bodies" of cancer and certain vesicular structures occurring regularly in the gametogenic, but not in the ordinary somatic, cells, may be found to hold good.

It was shown by one of us,* in 1895, that during the prophase of the heterotype (first maiotic) mitosis of the spermatogenetic cells, the archoplasm undergoes a highly characteristic and peculiar metamorphosis. In normal somatic, or pre-maiotic, cells the archoplasm is seen to lie beside the nucleus as a dusky mass of protoplasm in which are contained the centrosomes. That is, the attraction sphere consists of the archoplasm *plus* the centrosomes.

But during the prophase of the heterotype mitosis these constituents become separated. The centrosomes are found to lie *outside of* and detached from the archoplasm (see fig. 2, and also Appendix 5). At the same time the archoplasm itself undergoes a change. It becomes vesiculated, and finally, at the close of this cell generation, it is lost in the general cytoplasm of the daughter cells.

In the prophase of the second maiotic division (homotype) the same phenomena recur. When the homotype mitosis is over the constituents of the sphere, or at least some of them, enter into direct relation with parts of the spermatozoon which arises by further differentiation of the cell. As regards the archoplasm, with which we are more directly concerned, it is again seen to contain a number of minute vesicles which continue, as before, to grow in size, whilst each contains a single refractive and stainable granule (fig. 2). Subsequently, several of these vesicules fuse together, so that at a later stage in the metamorphosis of the cell into a spermatozoon there only remains a single large clear body, bounded by a distinct membrane, containing in the centre one or more darkly staining granules (fig. 3).

This body, originally described by one of us in 1895 as the archoplasmic vesicle, is a very conspicuous and apparently constant feature peculiar to the spermatogenetic cells of, at any rate, the vertebrata, and it has since been encountered beyond that group by other observers.

When fully developed it often assumes a size approximating to that of the nucleus. Indeed, the latter is often deformed and made to assume a crescentic or cuplike shape owing to the enlargement of the adjacent archoplasmic vesicle. The vesicle and its contents ultimately forms the so-called "cephalic cap" of the spermatozoon.

The remarkable similarity between the structure just described and those known as "Plimmer's Bodies" will have become obvious. It is not, perhaps, accidental that just as in the case of nuclear divisions, so also in the cellular inclusions, a parallelism between the cells of the reproductive tissues and of cancer cells should be found to exist. But

* Moore, 'Internat. Monatschr. f. Anat. v. Physiologie,' vol. 11.

we do not on this account regard the cells of cancer as *identical* with those of the sexual cells, as we were careful to point out in our first communication in 1903.

But the resemblances between what we have termed gametoid, and the true gametogenic cells now seem to be even more significant than they appeared to be at that time. Both classes of cells are autonomous to a very high degree, and both possess the faculty of continuous or intermittent multiplication independently of the tissue requirements of the organism. And finally both exhibit cellular and nuclear metamorphosis which not only, *mutatis mutandis,* resemble one another, but differ materially from those pertaining to the normal somatic cells.

It is possible that the malignant elements are the outcome of a phylogenetic reversion, as was suggested by Sir William Collins, but the matter is obscured by the disturbing influences that have been operative during actual ontogeny of the cells and tissues from which these elements have sprung. If this be so, the connection apparent between gametoid and the true reproductive cells will acquire a still deeper significance. But we propose to reserve the discussion of this question for another occasion.

In thanking those who have helped us with material we would mention especially Dr. Plimmer himself, who has most kindly placed preparations at our disposal.

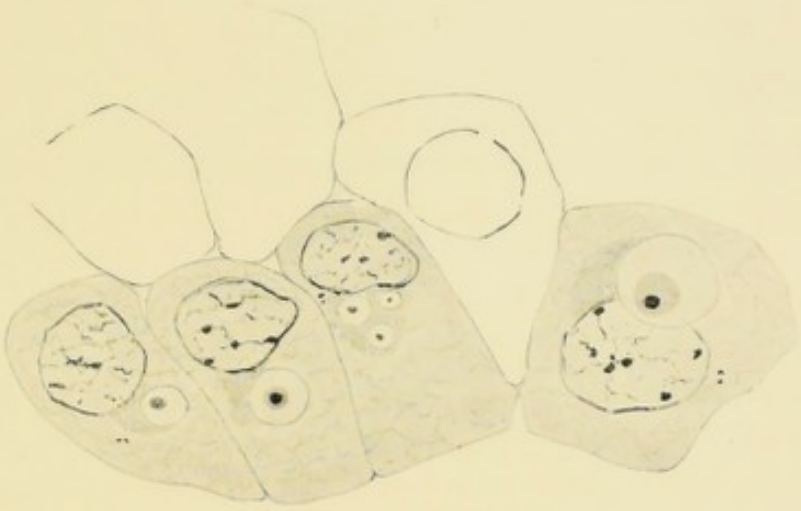


Fig. 1

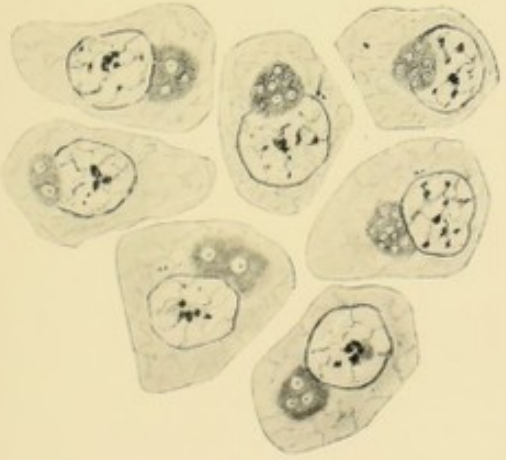


Fig. 2

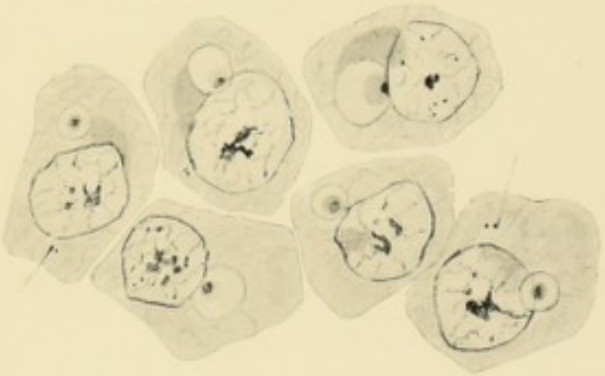


Fig. 3

To face page 3

3—ON THE BEHAVIOUR OF LEUCOCYTES IN MALIGNANT GROWTHS*

By J. E. Farmer, F.R.S., J. E. S. Moore, and C. E. Walker.

[From the Transactions of the Pathological Society of London, vol. 56, Part III, 1905.]

In the present paper it is proposed to give an account of observations relating to the peculiar behaviour of leucocytes in very early examples of carcinoma occurring in various parts of the human body.

The phenomena described appear to be mainly, if not entirely, restricted to cancer in its earliest stages, and not to occur in older growths, in metastases, or in grafts introduced into other individuals. Our observations at the present moment refer more especially to: (1) a small primary rectal tumour; (2) an early case of chimney sweep's cancer, and (3) an early epithelioma of the penis. In all of these the essential details in relation to the behaviour of the leucocytes are identical, and suggest that we are here dealing with cytological phenomena peculiar to the earliest phases of the transmutation of normal tissue-cells into cancerous elements.

It has frequently been noticed that around areas that are becoming cancerous there exists a marked activity among the leucocytes, and the fact that cells in a cancerous area may contain leucocytic bodies within their cytoplasm has frequently been observed. The latter cases have been regarded generally either as phagocytic invasion of the leucocytes or as an indication that the cancer-cells have assumed a phagocytic character with respect to the leucocytes. The results of the present investigations are not in accordance with either of these views. It was found in the rectal carcinoma above mentioned, for example (a growth that had hardly attained the size of a bean), that there was a very distinct zone of transition from the normal to the cancerous elements round the periphery of the tumour—that is to say, a zone in which it was possible to pass, within the thickness of a few cells, and almost insensibly, from elements that were merely actively dividing in the mucous layer to cells that had assumed most markedly malignant characters.

In this and other cases that we have examined we were irresistibly driven to the conclusion that the growth had not originated from a single cell, or even from a few cells, but was being evolved by the direct conversion of a great many elements definitely functioning as mucous cells into those of a truly malignant type.

Immediately within this outer zone of the tumour the leucocytic crowding was most strikingly apparent, and in a number of cells it was easy to discern the presence of leucocytes which had invaded the cytoplasm of the epithelium, where they stained readily as an inclusion. This phenomenon did not occur in the adjacent healthy tissue, and we have been unable ourselves to trace it in inflammation produced by artificial means. But the most important as well as the most singular feature about the intrusion of the leucocytes into the tumour-cells lies in the fact that neither the leucocyte nor the invaded tissue-cell appears to be injuriously

* For illustrations see Figs. 30 to 38 in body of Report.

affected. Neither appears to be eventually destroyed, and it was soon found that many epithelial cells that were in stages of active division contained leucocytes that had been engulphed in their cytoplasm. In many instances it was found, moreover, that while the tissue-cell was dividing mitotically, the included leucocyte was also dividing in a similar manner and at the same time. Both nuclei either passed through their mitotic evolutions upon separate spindles or the spindle figures become confused as is ordinarily the case in the first cleavage of the ovum.

It is, we think, clear from the observations just recorded that a mixture of the chromosomes derived from the leucocyte and tissue-cell respectively is distributed between the daughter nuclei resulting from the mitosis. In this way a complete disturbance of the normal chromosome constituents of the cell will be affected, and the distribution must be of a qualitative as well as of a quantitative character. It may be well to indicate that if this process be compared to fertilisation, there exists an important difference between true gametes and the cells concerned in this anomalous fusion. In the former (gametes) we are dealing with nuclei that have passed through the phases of reduction (maiosis), whereas there is no evidence at present to show that this is so in the case now under consideration. What its relation to the ultimate reduction that does occur in neoplastic cells may be, is a subject for further investigations.

It may be pointed out that the fusion here described in no way corresponds with that union which has been stated to occur between the definite cancerous cells of certain neoplastic grafts.

4—ON THE CYTOLOGY OF MALIGNANT GROWTHS*

By J. Bretland Farmer, F.R.S., J. E. S. Moore, A.R.C.S., and C. E. Walker.

(Received November 17,—Read December 7, 1005).

[From the Proceedings of the Royal Society, B, Vol. 77, 1906].

In the winter of 1903 we presented to the Royal Society a preliminary account of the results of our investigations on the cytology of malignant or cancerous growths, in which we stated that we had recognised a certain type of nuclear division, known as the heterotype, to occur in the cells of these pathological tissues. Hitherto, this peculiar kind of mitosis, characterised *by a reduction to one-half of the normal number of the somatic chromosomes,* together with highly characteristic forms of their bodies themselves, had only been known to occur in connection with the so-called reduction division, that, in animals, immediately precedes the formation of the sexual elements. These reduction divisions constitute a well-known phase in the life cycle of all higher animals and plants, consisting invariably of two consecutive stages, which are distinguished as heterotype and homotype mitoses respectively. As these two mitoses constitute so well-defined a stage in the cellular life history of the higher organisms, we have proposed to emphasise this fact by the introduction of the term "maiosis," designating the stage itself as the maiotic phase. Thus the heterotype and homotype mitosis form respectively the first and second maiotic division.

We propose, in the present paper, to deal more fully with the cytological details of malignant growths, in so far as we have been able to investigate them, and we shall endeavour briefly to point out the conclusions that we think may legitimately be drawn from them. In doing this we desire, as far as possible, to confine ourselves to a consideration of the evidence we have been able personally to obtain, and we propose to avoid a general discussion of the numerous theories as to the etiology of the disease, and especially of those that are based on clinical experience, except in so far as our own observations seem to throw light on those matters. We are fully aware that in pursuing such a course, we lay ourselves open to the charge of incompleteness, and of unduly neglecting the views of others. But an attempt to deal at all adequately with the vast literature that has grown up around the subject of cancer would involve a very lengthy, and probably not a correspondingly fruitful, discussion.

We further propose to confine ourselves to a consideration of carcinomata, and we do not intend to deal with sarcomata at this juncture, since we have enjoyed far fewer opportunities of examining growths of this class with anything approaching to completeness. On the other hand, we have been fortunate in securing a large number of carcinomata of very different ages, for which we are indebted to the kindness of several London Surgeons and others.

We have been able to study extremely early stages in cases of cancers of the rectum, scrotum, penis, lip and tongue. A similarity in all essential features was plainly apparent in the history of the cellular evolution of each of these growths respectively. It will, therefore, be sufficient for the purpose of this paper, and it will also conduce to brevity, if we confine

* The numbers of the figures mentioned refer to the illustrations in the body of the Report

our account, especially to one of them, merely premising that it represents a type to which the rest really conform in all essential details of interest in the present connection. We will, therefore, select as our chief example a very early case of rectal epithelioma. This was not only a very young, but also a very actively proliferating growth, and it was preserved immediately after excision.

For the sake of clearness it will be useful to give a short preliminary description of the mucous and subjacent layers of a healthy rectum as they appear under high magnification. The boundary of the lumen of the gut is composed of a large celled columnar epithelium, the elements of which are generally set one row deep upon the basement membrane. The latter is formed from the connective tissue and other elements of the submucous layer. All over the interior surface of the intestine the mucous epithelium is produced outwards into folds that bound correspondingly finger-like cavities, the latter forming the so-called crypts of Lieberkühn. As seen during ordinary states of activity, the epithelium is composed of elongated cells, each consisting of a mass of granular protoplasm in which a large nucleus is situated. The position of the latter in the cell is subject to individual variations. Amongst these resting cells are others in which the cytoplasm contains masses of secretion, and these constitute elements commonly known as goblet cells. In between the cells of such a rectal epithelium are always to be seen a few wandering elements which are apparently migrating from the submucous into the mucous layer and *vice versa*. Their number is, however, very limited, and they either resemble the white corpuscles of the blood, or their nuclei exhibit a lobed appearance which seems to represent a stage of fragmentation.

In the processes of the submucous layer which interdigitate with the crypts, we find, in the first place, a kind of framework or loose scaffolding of intestinal connective tissue, between the strands and sheets of which can be seen the loops of the anastomosing rectal arteries and veins. Within and without this region, but especially in the centre of the projections of the submucosa, vast numbers of lymph bodies are always conspicuous, the central mass or core of the latter marking the termination of the irregular lymph sinuses and vessels.

Amongst the columnar cells of the mucous layer during states of ordinary activity we occasionally encounter nuclei in various stages of ordinary mitosis. More rarely nuclei in process of fragmentation or amitosis may be seen. Neither kind of division is frequent, and the tissue seems to be merely regenerating itself by the replacement of individual cells as fast as these disappear. Amongst the leucocytes and lymph bodies, as well as occasionally amongst the connective tissue corpuscles, division is also plainly to be discerned. The nuclei of the connective tissue elements divide mitotically, whilst in the leucocytes we meet both with true mitosis and with those peculiar forms of leucocytic fragmentation that have already been described and figured by other writers, and are generally well recognised. Wherever we light upon true mitotic figures, whether in the mucous cells, in the connective tissue elements, or in the lymph bodies, the phases of division invariably agree with the type of mitosis characteristic of the non-reproductive portions of the body. They are typically somatic or premaiotic.* In every mitosis of this nature the chromosomes

* See Farmer and Moore, "On the Maiotic Phase (Reduction Divisions) in Animals and Plants," 'Quart. Jour. Micro. Sci.,' vol. 48.

emerge from the resting nucleus in the form of elongated or bent rods, and in the ordinary premaiotic number (32).

During the stage of the equatorial plate each of them is easily seen to be longitudinally split, the two halves passing respectively to the opposite poles of the spindle to contribute to the formation of the two daughter nuclei. Under ordinary conditions the above cytological conditions and appearances remain unchanged in the rectum.

Having completed that foregoing brief survey of the structures and changes that occur in the cells of a healthy rectum, we are in a position to consider the features that arise on the early development of a cancer in this region. In the case of the neoplasm we are especially concerned with, the area involved was very small, barely a centimetre in diameter, and this area marks the original seat of the disease. The central portions were but slightly ulcerated or broken down, whilst the margins were hardly at all raised.

Towards the periphery of the growth the columnar cells were scarcely displaced, but they exhibited a more or less altered appearance when compared with the still healthy cells in their vicinity. There could, in fact, be traced a narrow and not very sharp line of demarcation that distinguishes the cancerous from the non-cancerous epithelial elements. A consideration of the structure of the cells in this region makes it perfectly clear that the growth had not proceeded from a more remote centre to invade the healthy mucosa, but that the cells of this layer were themselves assuming the peculiar characters of the growth. In other words we are confronted with a primary transmutation of normal and functional cells into those of cancerous tissue. The tumour was small and flat, the change visible at its margin having presumably proceeded centrifugally over the more developed central area.

Thus the growth, regarded as a whole, must be considered as having originated from a relatively large number of functional epithelial cells by a direct conversion of them into neoplastic elements. No other interpretation seems reconcilable with the facts of the case, but we may defer the theoretical conclusion involved therein for subsequent consideration.

But, notwithstanding the evidence for the marginal spread of the growth by a direct alteration of the cells in this region, when once the change has been effected in them, the cancerous cells begin on their own account to invade the deeper layers of tissue situated beneath the epithelium. The general nature of the process of invasion is so well known as to call for no specially detailed description here. It will be noted, however, that the ingrowing cancerous tissue long retains many of the features characteristic of the particular epithelium from which it has sprung. This is, of course, not uncommon, especially in the case of glandular tumours.

The marginal zone of demarcation between the diseased and healthy tissue is distinguished at its periphery by a barely perceptible increase in the size of the elements that compose it. Immediately within this outermost limit a rapid multiplication of the cells is seen to be taking place, and even in the second or third cell from inside the margin the altered character is easily recognised. The cells exhibit an increase in cytoplasm, a comparative absence of secretory activity, and a peculiar and well defined change in the appearance of the nucleolus. The alteration in this last-named structure consists in its larger size and denser appearance. Furthermore, very many nuclei are to be seen in a state of active division. Whilst some exhibit various stages of mitosis, others are clearly undergoing fragmentation or amitosis.

At this stage of the development of the tumour, the peripheral cells that are dividing mitotically show all the characters of ordinary premaiotic divisions, and the normal number (32) of chromosomes can frequently be counted with certainty. But concomitantly with the first changes indicated in the epithelial cells at the edge of the neoplasm, a marked activity may be observed to take place on the part of the leucocytes. These bodies are seen to be in a condition of active migration and multiplication, much like that which occurs during the early stages of simple inflammation. In the subsequent stages, however, the early parallelism with inflammatory processes is lost, and there supervenes a remarkable phase in the further development of the cancerous cells. Not only do the cells of the tissue in question multiply with great rapidity, whilst the leucocytes amongst them are enormously increased in number, but the latter are seen not infrequently to force their way into the cancer cells, particularly in the so-called "giant cells," where, however, they are still to be recognised with ease and certainty (figs. 30 to 34). This circumstance has already been noticed by others, but we have been led to attach a somewhat special importance to its occurrence. Some writers have suggested that the cancer cells are acting phagocytically upon the leucocytes, but, as a matter of fact, the further sequence of events indicates that the cancer cells are no more to be regarded as attacking the leucocytes than the latter as destroying the cancer cells. There can be no possible doubt that the leucocytes actively force their way into the elements in question. They may not seldom be observed to be in close juxtaposition with these, or in a hollowed depression, or finally they may be discovered just within the cell membrane, where they are easily recognised on account of their characteristic nuclei. They show no signs of disintegration—at least, in the great majority of cases—and the fact that they may persist for a considerable time without destroying the cell into which they have invaded, is proved by examples in which a leucocyte lying in the cancer cell is seen to be surrounded by several nuclei that have clearly originated by the fragmentation of the original cell nucleus, and, indeed, one of these is shown to be still dividing amitotically.

But the strongest proof of the persistence of the leucocyte under these remarkable conditions is afforded by the cases, not few in number, in which we have been able to trace the leucocyte actually dividing within the cancer cell (figs. 35 to 38). Of course, it is only during the early stages that it is possible to be certain that a second dividing nucleus in a mass of protoplasm belongs to a leucocyte, and does not represent mitosis in a small nucleus that has arisen by fragmentation. But we have seen so many cases of early stages of leucocytic mitosis within the cancerous (or "precancerous") cell that it seems impossible to resist the inference that many of those frequently occurring cases in which a small nucleus is seen in the latter phases of mitosis within the large nucleated cancer cell are to be attributed to this source. The nuclei of the cancer cell and leucocyte often divide simultaneously, and the two nuclear figures may also coalesce more or less intimately, and thus a commingling of leucocytic and epithelial chromosomes occurs on a spindle that becomes common to the two nuclei concerned. The cells so affected were, as already stated, usually the very large (giant) cells so characteristic at this stage of the development of the tumour, and we found that more than one leucocyte might enter and persist in a single cancer cell. In the earlier stages, of course, there is no difficulty in clearly recognising the intruding cell, since it retains its own cytoplasm and limiting membrane intact, and the highly characteristic structure of the nucleus enables it to be identified even after these criteria have ceased to exist.

In the same region in which this series of events is proceeding a number of cancer cells are to be seen in various phases of mitosis, and, both in the aster and diaster of such nuclei, larger numbers of chromosomes were often encountered than are proper to normal somatic cells. These increased numbers are partly to be ascribed to the pluripolar mitosis distinguished by Hertwig and by von Hansemann, and they result from the simultaneous mitosis of a number of nuclei lying in a common cytoplasmic mass (see fig. 3).

But the observations recorded above indicate that, in the addition of lucocytic nuclei to those of the actual epithelial cells, we have confronted, at any rate, with one of the sources to which these excessive numbers of chromosomes (hyperchromatic nuclei of von Hansemann) may be attributed, although a large number of the cells continue to multiply in the manner already described, it may also be seen that there exists a very considerable amount of amitosis, or direct nuclear divisions in the cells of the young parts of the tumour. There appears to be no evidence which would point to the conclusion that amitosis is in any way bound up with degeneration, or diminishing activity in those cells in which it occurs. Elements that have previously multiplied by amitosis and by fragmentation have given rise to the highly characteristic multinucleate cells, may again assume the mitotic method of increase, and *vice versâ*. A curious feature in the further division of these multinucleate cells, or syncytia as they may, perhaps, be more appropriately termed, is seen in the almost invariable circumstance that, on the resumption of mitotic activity, all the nuclei are in exactly the same phase.

This simultaneous character of the process is one which is shared by many other syncytia, *e. g.*, the myxomycetes. In these organisms, the nuclei are commonly observed not only to be dividing simultaneously over a considerable area of the plasmodium, but they also exhibit identical phases of the process at any given time. In examples of this simultaneous mitosis within the neoplastic syncytia, it often happens that the spindles of some, or even all of the dividing nuclei, become more or less intimately fused together, and in this way various forms of pluripolar mitosis are produced. Probably these pluripolar divisions owe their origin chiefly to the cause just indicated.

The figures produced are extremely variable, and it not unfrequently happens that, whilst the chromosomes belonging to the different nuclei are aggregated in the centre, the poles of three or more of the spindles involved are quite separate. In other examples the groups of chromosomes do not coalesce, but each equatorial plate is quite distinct, and lies in a plane different from that occupied by the equatorial plates of the other spindles. But when a more intimate fusion of the ends of two or more spindles takes place, it is obvious that the daughter nucleus formed in relations to such unions will receive an excessive number of chromosomes.

We would call special attention to the fact that giant cells of this character, also containing several nuclei, are present not only in the normal human testis, but also in the so-called red bone marrow, and that pluripolor mitosis may occur in such cells in a manner precisely similar to that so characteristic of cancerous tissue. The divisions of these early cancerous cells also exhibit other characters likewise encountered in the cells of the testis. Very often the daughter chromosomes do not move regularly towards the poles, but some either stray out of the direct line, or in other ways occupy unusual positions. These figures are also well known to occur in the heterotype division of some spore mother-cells of plants.

In yet other examples of divisions in cancerous tissues, we have confirmed the observation of von Hansemann that some of the chromosomes, as they are passing to the spindle poles get ahead of their fellows, and form isolated or grouped chromatic particles that look as if they are about to be left out in the cytoplasm when the daughter nuclei become reconstituted. These figures are also paralleled by similar occurrences that may be seen in the cells of the testis, and they are known to occur during the maiotic divisions of some plants.

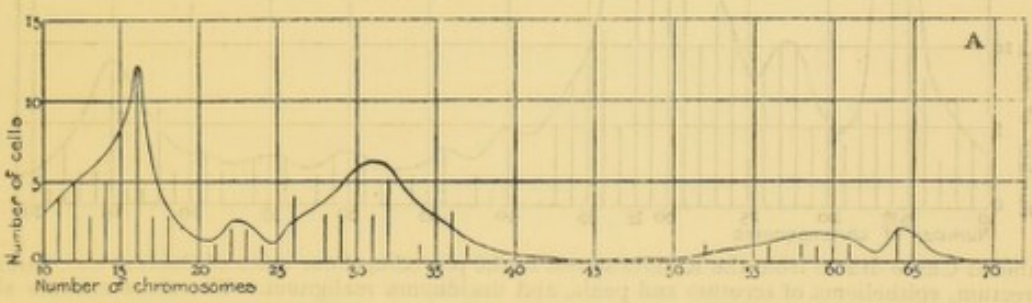
It is thus evident that hyperchromatic nuclei, that is, nuclei containing an excessive number of chromosomes, may be produced in at least two ways: firstly, by the inclusion of leucocytes, and the incorporation of the chromosomes belonging to these bodies with those of the cancer cells when mitosis sets in; secondly, through the formation, whether by amitosis or mitosis, of multinucleate syncytia, and by the subsequent confusion and mixing of the chromosomes originally belonging to two or more of the nuclei when the equatorial plate stage is reached.

These aberrant modes of division are found to proceed concurrently with the normal somatic mitosis that are going on in other cells in their immediate vicinity. It is impossible to say definitely whether there may exist any sort of alternation between the two types, though we are inclined to think that such is not the case. It is, however, important to notice that all the mitoses described above, whether they are normal in the number of chromosomes or not, agree in conforming to the somatic type of division. That is to say, no matter how many or how few the number of chromosomes involved may be, the spireme eventually divides into a number of rod-like elements, each of which splits longitudinally, and the daughter chromosomes resulting from such fusion are severally distributed betweeen the daughter nuclei finally produced. In such typical cases this of course means that each of the two daughter nuclei receives one longitudinal moiety of such original chromosome.

But as we pass inwards from the growing edge of the tumour we encounter cells in which the nuclei exhibit important deviations from the ordinary somatic type of mitosis, and exhibit the characters otherwise met with during the heterotype division (*cf.* figs. 9 to 25). In the early stage of the phase of such nuclei the spireme exhibits that characteristic bunched appearance recalling the well-known contraction figure that is normally to be seen at the onset of the maiotic phase, that is in the prophase of the heterotype mitosis, in animals and plants. In addition to this, we have been able to ascertain that at about the same stage the spireme thread exhibits the longitudinal fission (fig 19) that is highly characteristic, though perhaps not exclusively confined to the prophase of the heterotype division. The fission is especially well seen in those cases in which a marked polarisation of the spireme is apparent. But the most striking evidence of the validity of the comparison that we drew in 1903 between these particular nuclei and those of the reproductive cells during the maiotic phase of the animals and plants does not depend solely on the similar mode of evolution of the chromosomes from the resting nuclei in the "gametoid" cancerous and the true reproductive elements. The number of the chromosomes furnishes a far more important criterion. It is seen that a large number of dividing nuclei contains less than the normal complement of chromosomes. We have made a number of careful counts of the chromosomes in numerous cases of carcinoma, and always with the same result. In especial, we are indebted to Mr. L. Robinson for his assistance in this somewhat trying task. He has estimated the

chromosomes in 400 dividing nuclei, taken (100 from each) near the actively growing regions of three different carcinomata originating respectively from the rectum, scrotum, penis, and in an example of deciduomo malignum.

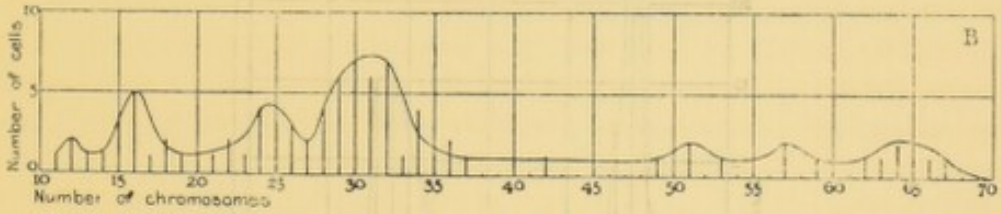
In every case we find two well-defined maxima, one set of nuclei containing 32, the other 16, chromosomes. For purposes of comparison he has counted chromosomes of the testis of the cockroach (Curve F), so as to obtain a control indicating the probable degree of accuracy represented by the estimations in the cancer nuclei. The same two maxima are, of course, apparent, but there is a similar average error around the maxima, due to the difficulty of the actual counting, and also the chance that some of the chromosomes might be absent from the section, or that a limited degree of variation may really occur. And, having regard to the fact that in the human species the chromosomes are not easy, even under favourable conditions, to estimate very accurately, whereas in the case of the cockroach the observer encounters far less difficulty in this respect, the results, may, we think, be described as satisfactory. For although, after what we have said, it is obvious that owing to amitosis, and especially to pluripolar mitosis, a considerable extent of variations is to be anticipated, the grouping of the numbers around the maxima of 32 (somatic) and 16 (reduced) is quite unmistakeable, as is shown in the accompanying curves.



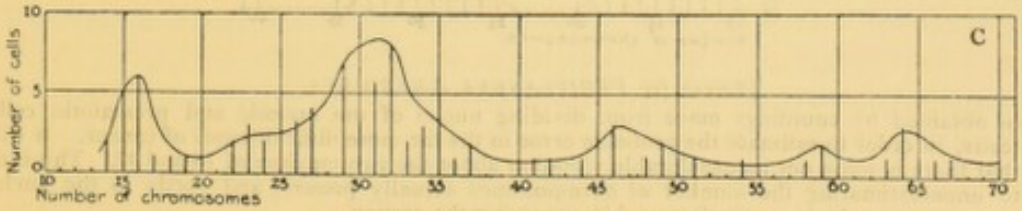
CARCINOMA OF RECTUM.

The ordinates represent the number of cells that contained any given number of chromosomes, as indicated by the abscissæ.

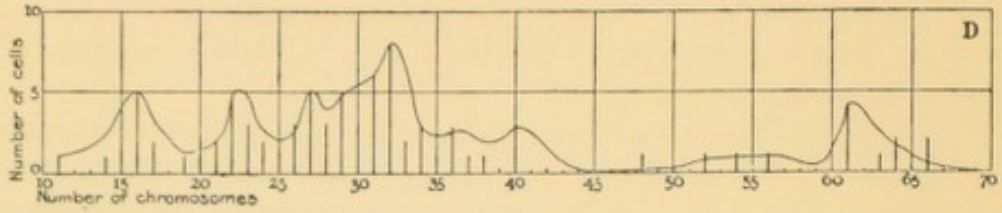
A. Cancer of the Rectum.—The hypochromatic nuclei to the left somewhat obscure, the maximum at 16. The grouping of numbers about 24 and 64 are fairly well shown.



EPITHELIOMA OF THE SCROTUM.



EPITHELIOMA OF THE PENIS.

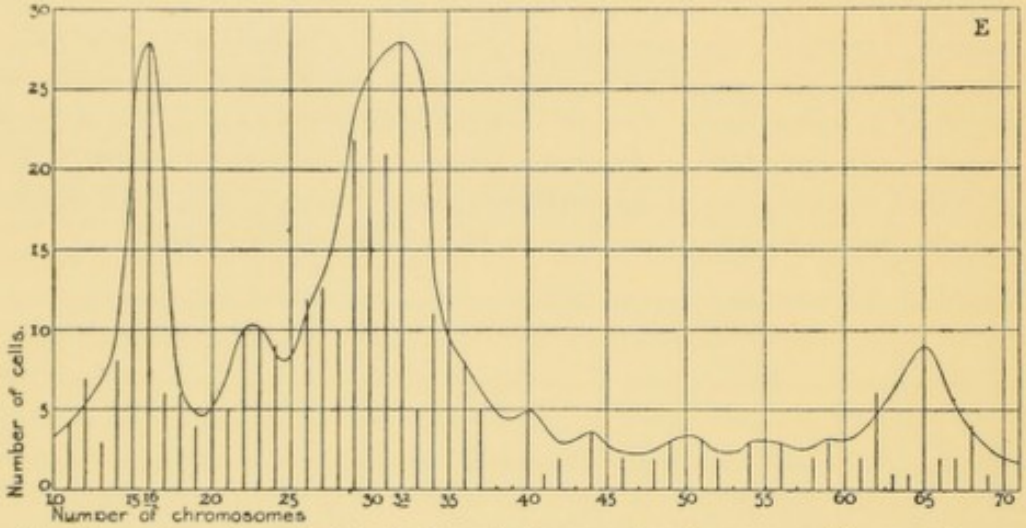


DECIDUOMA MALIGNUM.

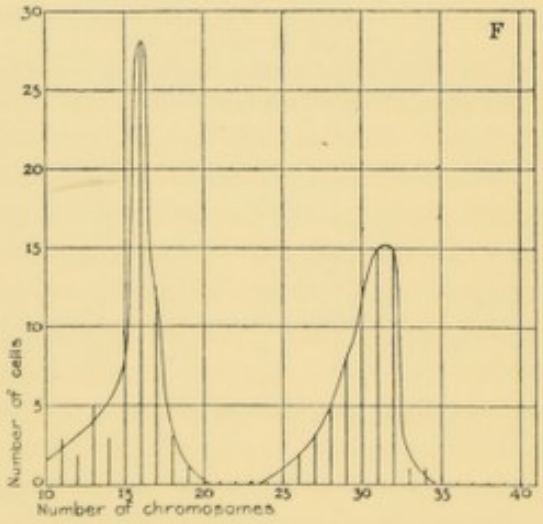
B. Epithelioma of the Scrotum—The maxima about 16, 24, and 32 are distinct, that about 64 not so clear.

C. Epithelioma of the Penis.—The maxima in the regions of 16, 24, 32, 48, and 64 are all fairly distinct.

D Deciduoma malignum.—There is considerable irregularity in the nuclei in this growth, which was somewhat advanced, and deviations are therefore to be anticipated.



E. Combined Curve drawn from the Results shown in the preceding Four cases of Cancer, viz., carcinoma of rectum, epithelioma of scrotum and penis, and deciduoma malignum. The three maxima about 16, 32, and 64 are unmistakeable.



TESTIS OF *PERIPLANETA AMERICANA*.

F. Curve obtained by countings made from dividing nuclei of the maiotlc and premaiotic cells of the testis, in order to estimate the probable error in the far more difficult cases of cancer. It will be seen that there is some not inconsiderable variation about the two maxima of 16 and 32. This is due partly to underestimating the number of chromosomes actually present, and partly to the nuclei having in some cases been partly damaged in preparing the section.

We shall further consider this matter in the concluding part of the paper: at present we are mainly concerned with showing that there exists a striking resemblance between what we have termed the "gametoid" cells of cancer and the cells of normal reproductive tissues, and as we pass to the later phases of mitosis we find the same loop and barrel-shaped chromosomes present in both, and we have occasionally seen, during the diaster of a cancer nucleus, the late longitudinal fission in the daughter chromosomes as they diverge from each other, just as it occurs in the heterotype diaster of so many animals and plants. An inspection of the curves shows the relative frequency of the different numbers of chromosomes met with in the younger cancerous areas. Whilst, as already pointed out, the two maxima of 16 and 32 are unmistakeable, it is also obvious that amongst the generally irregular numbers two other groups occur with greater frequency than others. Thus, there is a distinct indication that nuclei containing about 24 chromosomes may be regarded as forming a distinct group, also that a second, though far less well-marked, series is characterised by containing about 64 (double the normal somatic number) chromosomes. It may be that the latter are related to the ingression of the leucocyte already described, but it is difficult at present to guess at the significance of the grouping of 24. There is no obvious indication that the nuclei with 48 chromosomes are specially common, and in the absence of more direct evidence, it is useless to indulge in speculations that may prove to be devoid of all foundations.

In animals, as has already been stated, it invariably happens that, after the onset of the first maiotic (heterotype) mitosis, there ensues only one further nuclear division, commonly designated as the homotype, on account of its close general resemblance with a normal somatic mitosis. The principal point of constant difference lies in the retention in the former of the reduced number of chromosomes. The cells originating from this division give rise after a more or less complex series of changes of form and of the inter-relation of their constituent parts and the sexual cells without any further intervening nuclear divisions. In plants this is not the case. The cells issuing from the homotype mitosis always undergo one or (often) many subsequent divisions before some or all of the resulting units develop into sexual cells. It is therefore of interest to find in cancerous tissue that there is abundant evidence that the cells, the nuclei of which have undergone reduction, are capable of continued division, and, indeed, a great part of the tissue of the cancer is made up of such cells, which, in accordance with the terminology we have elsewhere employed, we may term post-maiotic, or "gametoid."

It will be seen that we differ from von Hansemann in our explanation of these "hypochromatic" nuclei, regarding them as having arisen, not as the author just named believes, by a dropping-out of chromosomes from the spindle, or through some form of degeneration, but chiefly as the result of a process resembling, or identical with, that by which reduction is ordinarily effected in the tissues destined to give rise to the gametic cells. But we desire to definitely state that, in using the term "gametoid," we expressly differentiate between the cancerous cells and those of normal reproductive tissues. The relation existing between them, if any, is at present obscure; and, though we think the resemblances, which will be still further emphasised by facts we are about to describe, are very suggestive, we are far from holding the views which have been expressly or implicitly ascribed to us by other writers as to the identityof gametic with "gametoid" cells and tissues.

Finally, then, it is clear that there exist in the facts of pluripolar mitosis, on the one hand, and in amitosis on the other, a mechanism sufficient to explain all the irregular numbers encountered in a young cancer. But the irregularities, while masking, cannot conceal the far more frequently recurring numbers of chromosomes, whereby the reduced (halved) and, though far less frequently, the double, numbers become apparent. But the existence of the irregularities indicated above often renders extremely difficult the task of deciding to what category a particular departure from the normal somatic number is to be relegated.

There is a further body of evidence bearing on the resemblance between cancerous and normal reproductive tissue to be derived from a study of the so-called "Plimmer's Bodies" of cancer.*

It was shown by one of us in 1895† that, during the prophase of the first maiotic (heterotype) division of the spermatogenetic cells of mammals, the archoplasm undergoes a peculiar and definite series of metamorphoses. In ordinary somatic or premaiotic cells, this body is seen to lie besides the nucleus as a dusky mass of protoplasm, in the centre of which are found the centrosomes. Thus, in these cells, the attraction sphere consists of the archoplasm *plus* the centrosomes.

When, however, we turn from the pre-maiotic or the somatic cells to the prophase of the heterotype (first maiotic) mitosis, we find these two constituents have become separated. The centrosomes migrate from the centre of the archoplasm, and are eventually seen to lie outside that body, and completely detached from it (see fig. 2 of Appendix 2). At the same time the archoplasm itself undergoes a change, small vesicles are developed in its substance, and, at the close of this particular cell-generation, both vesicles and archoplasm become merged and lost in the general cytoplasm of the daughter cells.

In the prophase of the second maiotic (homotype) mitosis the same peculiar phenomena recur, and the archoplasm and the vesicles, in like manner become lost during the later stages of this (homotype) division. In the spermatids, which result from it, the persistent centrosomes can be readily seen to be perfectly disconnected with the new archoplasm which is differentiated in these cells. The archoplasm becomes filled with minute vesicles, as in the two preceding cases, subsequently the vesicles enlarge, and they either fuse together, as in some mammals, or one usually takes the lead and grows larger than the rest, as commonly happens in the guinea-pig and in man (see fig. 3 of Appendix 2). The body thus formed was originally termed the archoplasmic vesicle in 1895,‡ and it is a very conspicuous and constant feature peculiar to the sperm cells of the vertebrata, whilst it has also been encountered by various observers in animals outside that group.

When fully developed, the archoplasmic vesicle often assumes a size approximating to that of the nucleus itself, the latter being often deformed into crescentic shape, owing to the enlargement of the vesicle that lies adjacent to it in the cell. In normal spermatids, the vesicle and its contents ultimately form the so-called "cephalic cap" of the spermatozoon (fig. 16, *a*).

* See 'Proc. R. S.,' vol. 76 B, "On the Resemblances existing between the 'Plimmer's Bodies' of Malignant Growths and certain Normal Constituents of Reproductive Cells of Animals," by J. Bretland Farmer, J. E. S. Moore, and C. E. Walker.

† Moore, 'Internat. Monatschr. f. Anat. u. Physiol.,' 1894.

‡ Moore, *loc. cit.*

Now, the "Plimmer's Bodies" are well known in the cells of many cancerous growths (see fig. 1 of Appendix 2), and they are most commonly met with in the young growing portions of the tumour. They appear in the form of vesicles, and consist eventually of a fairly well-defined wall, enclosing a clear space, in which is suspended a small and densely refracting granule. They appear to occur with greater frequency in cancers of a glandular or glandular-epithelial origin.*

They lie in the cytoplasm of the cancer cells, usually in close proximity to the nucleus. They vary in size from excessively minute bodies to forms as large as the nucleus itself. The special interest attaching to the "Plimmer's Bodies" depends on the fact that they have commonly been regarded as peculiar to cancer cells, although Honda† believes that he has occasionally encountered them in inflammatory tissue. They have, in fact, been variously interpreted. Some investigators have regarded them as parasitic organisms, more or less intimately connected with the ætiology of the disease, whilst others have seen in them a differentiation of the cancerous cell itself. Borrel‡ suggested that they might represent hypertrophied centrosomes, but the observations of Benda,§ who showed that centrosomes and "Plimmer's Bodies" co-existed in the same cell, have rightly been held to disprove the view advanced by Borrel.

When the foregoing facts are all taken into consideration, the case originally upheld by ourselves‖ appears to be a strong one. We see no escape from the position that the "Plimmer's Bodies" of cancer represent the archoplasmic vesicles that occur in the normal reproductive cells at the stages already indicated. And this forms an important link in the chain of similarities connecting cancerous tissue with the normal reproductive elements. But in this relation it is of interest to note that we have recently observed bodies, which appear to be closely similar to archoplasmic vesicles, to occur at apparently definite stages in the life history of certain leucocytes which are present in bone marrow.

GENERAL CONCLUSIONS.

To sum up the observations already recorded in this paper, it may be seen:—

1. That a primary growth originates in the first instance as the result of a change in the nature of a number of previously functional somatic cells.

2. That the transformation may affect a considerable number of cells, and certainly continues to operate for some time.

3. That, as a result of the change, mitotic and amitotic activity is awakened, and proceeds rapidly, with a consequent increase in the mass of affected tissue.

4. That during this increase a remarkable activity prevails amongst the leucocytes, at first resembling that seen in inflammatory processes, but finally leading to the union of at any rate, some of the affected cells with one or more leucocytes.

5. That in the subsequent divisions of these cells the nucleus of the leucocyte divides simultaneously with that of the cancer cell, and their chromosomes may become mingled in cleavage figure.

* Greenough, 'Third Report of the Caroline Brewer Croft Cancer Com.,' Harv. Med. School, 1905.
† Honda, 'Virch. Arch.,' vol. 174.
‡ Borrel, 'Ann. Inst. Past.,' vol. 15.
§ Benda, 'Verh. deutsch. Gesellsch. f. Chir.,' 1902.
‖ 'Proc. R.S.,' vol. 76 B, pp. 230 *et seq*

6. That multinucleate cells (syncytia) may arise by mitosis or amitosis, unaccompanied with the division of the mass of protoplasm.

7. That the resulting nuclei may divide normally and mitotically, or the nuclear figures may be more or less mingled, and hence all sorts of variations in the number of chromosomes may occur. But the mode of chromosome evolution and division follows the somatic type.

8. In addition, a form of mitosis occurs, leading to nuclei with half the number of somatic chromosomes, and the phases closely accord with those observed during the heterotype (first maiotic) mitosis of animals and plants.

9. Subsequent divisions occur, in which the reduced number of chromosomes is retained, the type of division otherwise resembling that of ordinary somatic cells. These mitosis fall into the category corresponding with the post-maiotic mitoses of plants.

10. During the maiotic and post-maiotic divisions in the cancerous cells, structures are present which have been designated as "Plimmer's Bodies." These are common to cancerous cells and to the reproductive cells of the testis at a particular phase in their evolution. The only other cells in which structures resembling the bodies in question have been observed are possibly those forming certain of the leucocytes in bone marrow.

It will be evident from the above summary that the change from the healthy to a cancerous development is intimately bound up with definite change in the cells affected. The onset of the change is probably to be attributed to the operation either of new stimuli upon the body cells, or to a change in the constitution of the latter. Such an alteration might originate in a variety of ways. For example, it might be ascribed to the influence of a parasite. But we have never succeeded in tracing any such cause, and it becomes necessary therefore to seek for some other explanation for the phenomena actually witnessed.

It is quite certain, in the first place, that we are dealing with the transformation of functional somatic cells into cancerous ones, and this, to our own minds, affords a complete refutation of the hypothesis as to the persistence of "embryonic rests," such as have been supposed by Cohnheim and his followers to account for the incidence of the disease.

We have drawn attention to the events that occur in connection with the invasion of the cells of the young growths by leucocytes, and, although we are fully aware that further investigations into the details of these processes are required before a final opinion can be expressed as to their true significance, the facts themselves are very suggestive.

Furthermore, the interest attaching to these fusions is not lessened by a study of the bone-marrow, in which the leucocytes can be most advantageously observed. For we have seen in this tissue all the abnormal types of nuclear and cellular division that are so highly characteristic of cancerous cells, and we have ascertained a fact of even greater importance, namely, that some of the nuclei of dividing marrow cells certainly possess less than the full complement (32) of somatic chromosomes. We would, further, lay emphasis on the occurrence, in the same preparations of bone-marrow, of other cells in which the process of mitosis was strictly somatic in character, both as regards the form and the number of the chromosomes. But it is none the less certain that the other nuclei exhibit chromosomes of a remarkable form, elongated in the direction of the spindle, and strongly resembling those which are so characteristic of the heterotype mitoses of the testis or of a cancer.

Whilst it is obvious that further investigation on the cytology of bone-marrow is urgently needed, it is evident that, if it should ultimately prove that the cells which are derived

from the results of fusion of a leucocyte with a tumour cell really represent the progenitors of the malignant elements themselves, a satisfactory explanation would be afforded not only of the striking nuclear character of the diseased tissues, but also of the invasive and destructive powers they undoubtedly possess. The destructive action of the leucocytes themselves on other cells of the body, especially during old age, is too well known, owing especially to the valuable researches of Metschnikoff, to call for further comment here.

Such a view of the case as is here tentatively suggested is not in conflict with the idea embodied in the term "gametoid" tissue, but rather forms an extension of it. We have, as already pointed out, from the first maintained the existence of a resemblence, extending to extraordinary minute detail, between the "gametoid," cancerous, and the reproductive tissue, which, in the case of animals, gives rise to the gametes immediately after maiosis. But it is also now certain that there exist certain striking similarities between the leucocytic and reproductive cells which are, in themselves, highly suggestive, and this is not diminished by a consideration of the earlier phylogenetic history of wandering and reproductive cells in more primitive animals, for example, in sponges.

For the present, however, and in the absence of more complete and accurate knowledge on the evolution of the leucocytes, we may close by remarking that the various peculiar characteristics of cancerous cells, find their closest analogies in the cytological processes that are exhibited in the formation of the reproductlve cells, and in those maiotic phenomena that so especially distinguish them.

5—THE MAIOTIC PROCESS IN MAMMALIA.

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[From the Thomson Yates Reports, University of Liverpool.
Williams & Norgate, Henrietta Street, London, W.]

The objects of the present communication are to give as complete and accurate an account, as our present methods will permit, of the maiotic phenomena in mammals: that is to say, of the successive changes which take place during the transformation of pre-maiotic, or somatic, into sexually reproductive cells.

The reasons which make such an undertaking desirable are numerous. In the first place, the existing observations bearing upon the matter are at present widely scattered, both as regards the portions of the maiotic process they refer to, as well as with respect to the types from which the illustrations have been drawn. In fact, curious as it may seem, so far as we are aware, no complete account of the maiotic phenomena in any mammal exists at the present time. The researches of Meves, Borst, Ebner, ourselves, and others have dealt with individual sections of the process, with the origin of the archoplasmic vesicle; the history the centrosomes in relation to the Spermatozoa; the existence of the heterotype and homotype divisions, and the like. But in no existing publication has the whole maiotic process been followed out. In the conjoint work by one of us* and Professor Farmer, the maiotic divisions in mammals were briefly referred to, as coming into the general scheme we were then able to formulate regarding the maiotic process in animals and plants; but the matter was necessarily dealt with in the briefest possible manner, and no attempt was made to indicate in detail the wide divergence which exists between the new interpretation of the processes involved, and the older conceptions regarding the heterotype division, contained in the works of Flemming and others.

It would thus not be undesirable that a full account of our observations upon mammalia should be published merely as a supplement to our former work. But beyond this, the fact that certain features peculiar to the maiotic change have been found by us† to occur as constant cytological peculiarities during the development of malignant growths in man, renders the details of the maiotic change in mammals particularly important at the present time. The formation of archoplasmic vesicles in mammalian sexual cells deserves special attention, for these very definite objects have been shown to be characteristically present in the cells of malignant growths.‡ Again it has been ascertained, as will be seen in the sequel, that during the maiotic change peculiarities occur in the position and behaviour of the centrosomes,§ and these peculiarities have also been shown to characterise the development of cells during the formation of malignant growths.

* The Maiotic Phase (Reduction division) in Animals and Plants. 'Quart. Journ. Micro. Sci., Vol. 48, 1904.

† J. B. Farmer, J. E. S. Moore, and C. E. Walker. 'Proc. R.S.,' 1903.

‡ J. B. Farmer, J. E. S. Moore, and C. E. Walker. 'Proc. R.S.,' 1905.

§ See also J. E. S. Moore. *Int. Monat. f., Anat. u. Phys.*, 1894.

Note.—At the last moment we have received a copy of a paper by Prof. F. A. Jenssens, "Evolution des Auxocytes Mâles du Batracoseps Attenuatus." We regret that it is at present impossible to deal with this now, though it will be seen that we differ from him materially upon some important points. At least one important stage described by us has not been recognised by Prof. Jenssens.

Thus in many ways it is desirable that a detailed account of the maiotic phenomena in mammals should be available, and the present memoir contains that part of such an history as is afforded by the study of the development of the male sexual cell. In a future publication it is intended that the development of the female sexual cells shall be similarly treated. But it may be pointed out that although the maiotic phenomena in the case of eggs generally, is vastly more difficult to follow, little, if any, new light will really thereby be thrown upon the subject. The broad features of the development of the egg in mammalia is sufficiently well-known to show already that its history will in all its cardinal features be parallel to the history of the male cells themselves. The only important matter to be readily obtained through an investigation of maiosis in female mammals will be in relation to the probable similarity, or identity, of certain of the so-called yolk-nuclei with the archoplasmic vesicles, or Plimmer's bodies, in the male. This matter is at present obscure, on account of the fact that bodies which are unquestionably of diverse origin, are included under the term yolk nucleus, and have not yet been sorted out into different and specific categories.

In dealing with a class of organisms so closely knit together as the mammals, it is not to be expected that there will be much diversity or important differences in the maiotic phenomena of different types, and so far as we know the whole course of events could be successfully illustrated through an examination of any single form. We have ourselves examined the maiotic process in men, baboons, bats, dogs, cats, rabbits, mice, guinea-pigs, hedgehogs, bulls, and the duck-bill platypus. On the whole, among these types, we have found that the guinea-pig is in many ways the best. Yet, as will be seen in the sequel, there are certain stages in the maiosis of this animal which are better illustrated by other types, consequently in what follows we have used the guinea-pig as a mammalian example perhaps best suited to illustrate the maiotic phenomena of the group; but have supplemented this example when necessary by observations based upon the examination of other forms.

For the sake of convenience we have in this, as in our former conjoint work,* considered the development of the sexual cells, as naturally divided into three stages:—Pre-maiotic, maiotic, and post-maiotic. The *pre-maiotic period* includes all that series of cell divisions which extends from the first segmentation of the egg to the prophase of the first maiotic (heterotype) division. The *maiotic period* embraces the heterotype and homotype divisions, and the intervening rest; while in mammals, as apparently in all animals, the *post-maiotic period* is only represented by the resting condition of the spermatid, or the egg after the second polar body has been extruded, there being in animals apparently no such post-maiotic divisions as generally occur in plants.† By this means we have, as explained in our former work, a consistent and simple terminology which can be applied with equal precision in the case of any animal or plant. At the same time we entirely obviate the necessity of using the cumbrous nomenclature which has grown up in the literature dealing with this subject, and according to which we find corresponding elements designated by the terms—Spermatocytes of the first and the second order; Ovicytes of the first and second order; Sporogonia; Oogonia; Spore mother cells; Spores; and the like.

* *Loc. cit.*

† See Appendix 8. C. E. Walker. "Observations on the Life History of Leucocytes." Proc. R.S. B, vol. 78, 1906.

I.—THE PRE-MAIOTIC PERIOD.

For some time after the first segmentation of the egg the cells whose descendants are destined to give rise to the sexual elements, or to form some portion of the animal body, are so far as their cytological characters go, all essentially similar. As regards the peculiarities of their divisional phenomena, whether the so-called somatic and reproductive tracts become differentiated early, or late, these peculiarities remain constant till the maiotic process is initiated in the reproductive series. Or, as sometimes happens through the appearance of cancer, and under exceptional conditions among plants, maiosis is produced within the elements of the somatic stock. Thus the pre-maiotic sexual cells may be viewed as undifferentiated somatic cells; or conversely, and probably more correctly, the so-called somatic elements can be regarded as differentiated pre-maiotic cells.

All the divisional phenomena of the pre-maiotic cells of the early sexual tract could be equally well illustrated by observations directed upon the ordinary somatic cells of the body, or *vice-versâ*. And, nothwithstanding the fact that the cells of the soma may become specialized, we regard all pre-maiotic elements, whether belonging to the soma or to the anlagen of the reproductive glands, or to the mature reproductive glands, as being essentially similar from a morphological point of view. Maiotic, as well as pre-maiotic, cells may become temporarily specialized; when, for example, archoplasmic vesicles, chromatic bodies, and other structures are present. And post-maiotic cells may become similarily histologically differentiated, as in the case of the spermatozoa. A bundle of spermatozoa is a mass of cells, each of which is more specialized histologically than a cartilage cell, or a connective tissue element, or even most gland cells. Again, the ordinary somatic functions may be discharged by post-maiotic cells, as in the case of the prothalli of ferns, and the peculiar morphological characters of the prothallus can be assumed by pre-maiotic cells in special cases.*

Consequently from such a point of view as the above the question of somatic or histological differentiation, loses all significance. What remains important are the constantly recurring features of the life cycle of the cells of animals and plants. The three phases, pre-maiotic, maiotic, and post-maiotic: to one of which the cells of any particular tissue invariably belong.

From this point of view the cells of the soma of any animal, and those of the young reproductive series, are both similar, in that they are both pre-maiotic in phase.

In mammals (in vertebrates and invertebrates for that matter) the pre-maiotic cell consists in its simplest form of a nucleus, and its associated cytoplasm (figs. 1 and 9). During rest the nucleus consists of a coarse and irregular network, or foamlike structure, composed of a denser substance (linin) within the shreds and patches of which are scattered in an irregular fashion chromatin granules. In the spaces between the strands of this composite network lies a less stainable substance, and there is generally at least one large, round mass of staining material, forming the so-called nucleolus. The whole nucleus during rest is bounded by a definite membrane.

* Digby, Miss L. 'Proc. R.S.,' 1905. The possibility of this happening also in the case of certain tissues in animals is also suggested by the observations recorded in Appendix 9.

The cytoplasm consists of a less refractive cytoplasmic mass surrounding the nucleus, and upon ultimate microscopical analysis this substance is found to consist of a finer foam-like structure, or net-work, between the strands of which there lie granules of various and non-constant properties and sizes.

In mammals, and many vertebrates at any rate, there can usually be differentiated in the resting cell a small patch of denser cytoplasm, generally near some portion of the nuclear membrane. Within this there usually appear two, small, highly refractive, bean-shaped bodies, which under certain conditions stain distinctly, and differ from the other granules to which allusion has been made in that they remain constant in relationship and appearance within the resting cell. These bodies are the so-called centrosomes, and the denser cytoplasm which surrounds them may be conveniently described by using Boveri's term of archoplasm; the archoplasm and centrosomes together being often spoken of as the attraction sphere (fig. 9).

Centrosomes are, however, not always present in cells. They appear to be constant in the tissues of higher animals, and among the lower plants; but they are not present in the cells of many higher plants, either during rest or during the active stages of division. They appear in those plants which possess them, among the Protozoa, and in maiotic cells, as we shall see, to be specially related to motile organs, such as Flagellae. And in general it may be said that the more primitive the type of cell, the more prominent the centrosomes will be. We are, at any rate for the present, inclined to regard them with van Beneden as primitive cell organs; but organs which in numerous instances have become lost.

All the pre-maiotic cells of the body, or indeed those belonging to any part of an animal except the adult sexual glands, multiply chiefly by mitotic division, for although amitosis is present in certain tissues, it is often resolvable, as in the case of Leucocytes, into mitosis, which is, as it were, hurried over, its mitotic origin being still in evidence. True amitosis is relatively rare, and at present obscure, both in its relationships and occurrence. Here again the primitive appears to be the more complex. The segmentation of the egg is produced by mitotic division. The fission of the Protozoa has been shown to be normally mitotic; while amitosis appears in the glands, and highly specialized tissues of the bodies of animals and plants.

From the first segmentation of the egg, to the definite formation of the sexual glands, the multiplication of the cells forming the whole body is carried out by a series of mitosis, which are all of a similar and pre-maiotic character; and a pre-maiotic division in a mammal may be described as follows:—

PRE-MAIOTIC DIVISION.

When a pre-maiotic cell is about to divide the nucleus becomes finer in texture, and more readily stainable. The coarse granules disappear, and are replaced by evenly distributed, finer, particles. The alveolar structure of the nucleus becomes subsequently coarser and coarser, so that the lining eventually takes on the appearance of a much-contorted ribbon, or ribbons, along which the chromatin is spread (fig. 2.) This figure, the so-called spirem, does not follow, as it were logically from the breaking up of the preceding network; but the nuclear contents assume their new form in an arbitrary fashion; for which at present we cannot account. In some cases the spirem has the appearance of a

coiled and endless filament; in others this is never fully attained. In any case, however, the spirem stage is succeeded by a phase in which the thick thread work has broken up into a number of segments, as in fig. 3. In all animals and plants the number of such segments, or chromosomes, is apparently constant, for any particular form, and in mammals this number is thirty-two in man, rats, and guinea pigs, twenty-four in mice.

While the above intra-nuclear changes are going on, others proceed in the cytoplasm. The centrosomes become more conspicuous, and are surrounded by fans of radiations, while eventually they separate from one another with great rapidity, and take up positions on opposite sides of the nucleus. Sometimes in the pre-maiotic division of mammals it is at this period observable that the chromosomes are apparently split longitudinally; but this cannot generally be seen until a later stage. At about the same time the nuclear membrane becomes irregular and disappears; the clear nuclear substance often remaining as a light space round the liberated chromosomes.

The radiations round the centrosomes rapidly increase, extend across this intervening space, and become fixed upon the chromosomes themselves, these bodies gradually taking up an equatorial position, still adhering by their middles to sheafs of spindle fibres (fig. 5.) At this period in the guinea-pig it becomes possible to make out that the bent, rod-like chromosomes are longitudinally split, and as the mitosis proceeds the two halves of each chromosome are gradually drawn away from one another towards the centrosomes (fig. 4.) Here they collect in a couple of irregular masses of V shaped daughter elements, which are at first arranged in the form of rings (or diasters), the centrosomes lying immediately beyond them (figs. 6 and 7.) In a short time the daughter chromosomes becomes vesiculate, and run into one another, so that eventually they form an irregular nuclear mass at either pole of the dividing cell (fig. 7.) These daughter nuclei become eventually surrounded by new membranes, and the centrosomes pass along a groove round their surfaces towards the original equatorial face of each. At the same time the whole cytoplasmic mass of the dividing cell becomes hour-glass shaped, and this character increasing, the cell divides into two, a small residuum of the substance forming a portion of the spindle which originally intervened between the separating chromosomes remaining visible in either cell, and apparently going to form the condensed mass round the centrosomes, or archoplasm (fig. 9.)

The nucleoli of such pre-maiotic cells become altered in appearance during division, sometimes breaking up into fragments, which disappear in the cytoplasm. In other instances they persist, and are visible in one or both of the daughter cells for a considerable time. After the daughter cells separate, the nuclei gradually pass back again by insensible degrees into the condition of complete repose originally possessed by the parent form (fig. 8 and 9.)

Such, then, is the character of the pre-maiotic cell division of mammalia. It is a process by which the permanent cell constituents, nucleus, sphere, and cytoplasm, are accurately halved, and their sundered moieties eventually reconstructed into two similar elements in the place of one. Substantively each daughter cell is a miniature of the original parent, and the process of division may go on rapidly, as during segmentation, so that the ultimate products are highly reduced images of the original egg; or as during the later growth of the embryo and during the replacement of tissue in the adult body, each daughter of division may grow again to its original size before a subsequent mitosis.

OUTLINE OF THE DEVELOPMENT OF THE MALE SEXUAL CELLS.

In the male sexual glands of mammals before the maiotic process intervenes, and throughout the rest of the body, except in those abnormal cases where malignant growths are superimposed upon the ordinary course of development, the elements which fill up the testicular tubules have been produced from the original blastomeres by repeated divisions of the above within maiotic type. During the first onset of the maiotic change cells lying within the mass filling up the tubules pass into the maiotic condition, and these are eventually discharged as Spermatozoa. The tubules, when mature, become converted into hollow pipes of connective tissue, lined inside with a single layer of pre-maiotic elements. These latter, by continuous divisions, give off internally layer after layer of cells that pass immediately through the maiotic change, eventually become shed into the lumen of the tubule as mature spermatozoa.

In an adult tubule there are, however, besides the layer of pre-maiotic sperm-producing elements, cells which are of a different character. These are the so-called foot cells, and although there origin is by no means as clear as it might be, their function is undoubtedly connected with the maintenance of future crops of Spermatozoa.

In the adult mammalian tubule the cells of the pre-maiotic layer have ill-defined walls, and in some instances it is suggested that the whole of this layer consists in reality of a nuclear syncitium rather than a pavement of individual cells. Moreover, in portions of this layer there exists nuclear figures, which certainly suggest amitotic budding. That is to say, appearances which would seem to indicate that at certain times the cells of the pre-maiotic layer are multiplied by amitosis before passing on into the later stages. The suggestion of amitosis in the pre-maiotic layer in mammals is of great interest from a theoretical point of view, and it is in conformity with appearances observed during the study of the periodical maturation occurring in amphibia.* We would, however, at present go no farther in this matter, as the work upon amphibia is incomplete.

In the adult mammalian tubule we have then a hollow pipe, lined inside with a layer of pre-maiotic sperm cells, and between these are interspersed a number of foot cells, represented red and grey respectively in figures on Plate VII. On the inner side of this layer there always exist numbers of older elements, which in the figure have been left uncoloured, and will be dealt with later. In such a tubule it will be found that at certain points individual cells, and patches of cells belonging to the pre-maiotic layer, are undergoing mitosis, and that this division is always of the pre-maiotic type. Moreover it can often be seen that at each such division of the pre-maiotic elements one of the daughter cells passes somewhat inwards towards the interior of the tubule, and then enters immediately upon the maiotic stage (fig. 55). Not infrequently cells belonging to this series are seen which contain one, two, or even four nuclei, and it seems probable that the syncytial mass of cytoplasm has not divided up along with the nuclei: multi-nucleate masses being the result. This inner layer of nuclei coloured blue (figs. 55 and 56) grow at first slowly in size relatively to the elements in the pre-maiotic layer. Subsequently, however, they grow much more rapidly, and at the same time they assume a distinctly chromatic and granular

* These observations are contained in a paper not yet published by Miss Embleton. The material was, however, worked out in the laboratories of the Cancer Research, Liverpool University.

appearance; this change in reality marking the onset of the maiotic transformation. Such cells are, as a matter of fact, entering upon the long prophase of the first maiotic heterotype division (fig. 56). When these cells have obtained the dimensions represented in fig. 56 they divide and give rise to daughter elements lying in the position represented in fig. 57. Subsequently the latter elements again divide, producing the second maiotic (homotype) division. These last products occupy the position represented in fig. 58, and without further division are transmuted into groups of spermatozoa, their connection with the foot cells becoming at the same time obvious (fig. 58).

Such then is a brief outline of the stages in the maiotic transformation as it occurs in the adult mammalian tubule during Spermatogenesis, and it remains for us now to consider in detail the various maiotic phases.

THE FIRST MAIOTIC (HETEROTYPE) DIVISION.

The nuclei which are about to enter upon the prophase of the first maiotic division become finally granular and chromatic, and after at first containing one or two large nucleoli* are seen to possess several scattered about the interior of the nucleus. Towards these chromatic centres the chromatic reticulum is curiously centred, as in fig. 10.

In mammals the appearance and arrangement of these chromatin centres is highly remarkable and interesting. They correspond to the structure which in 1904 we called the "chromosome anlagen," visible in the rest, and prophase of the pre-maiotic cells of *Periplaneta*. They are also similar to the bodies (Pro-chromosomes†) subsequently described in the first maiotic prophase by Strasburger, Miyaki, and Overton in a number of plants.

The results of our fresh observations upon mammals do not agree with those of the three last-named authors upon plants. They are, on the other hand, exactly in accord with and merely extend those already published by us in conjunction with Prof. Farmer upon the maiotic process in general (*loc. cit.*). The present detailed re-investigation of the synaptic phases in mammals being closely parallel with the similar observations recently published by us in relation to the synapsis in *Triton*.

In the earliest condition in which it is possible to distinguish the cells that are passing into the maiotic phase, from those of the pre-maiotic layer, it is found that the neuclei have acquired a closer and more chromatic reticulum. Within each there are to be observed several chromatic centres (fig. A). In many cases it is seen that the chromatic centres are double, or treble, or made up of even more parts of the same size (fig. A).

From these chromatic centres, the thread work of the nucleus radiates into its substance; and it can often be seen that from each chromatic centre two threads wander off into the general tangle of the incipient spirem. In the cell represented (fig. A) it will be seen that the number of the components of the chromatic centres varies from 1—5.

In a very large proportion of such cells, in the guinea pig, it is, however, found that the number of chromatic centres is 8, and that each is made up of two parts. This gives to the centres an appearance of duality which is undoubtedly that referred to by Strasburger and his pupils.

* Farmer and Moore, "On the Maiotic Phase (Reduction division) in Animals and Plants." 'Quart. Journ. o. Sci.,' Vol. 48, 1904.

† See Strasburger, Miyaki, and Overton.

Shortly after the stage just described the chromatic network, or fine spirem, contracts into the well known synaptic figure represented in fig. B; and this contraction seems to be brought about by the chromatin centres migrating to one side of the nucleus as is seen in the figure.

In both this and the earlier stage the whole of the nuclear threadwork is seen to be centred upon the chromatin bodies, and these become massed together in the manner represented in fig. C. In figs. B and C the spirem is seen to be also gradually unwinding; or opening out, into the characteristic loops shown in figs. C and D.

Now through the later stages it is not difficult to persistently follow these loops until we see them finally each become individually converted into one of the synaptic gemini (heterotype chromosomes); and since the number of gemini in the guinea pig is sixteen, it follows, there being usually 8 chromatic centres, that these latter each represent the ends of a couple of the sixteen loops.

Their frequently double appearance is due in the first place to the fact that at this stage they tend to be aggregated in pairs: although subsequently they become all dissociated, and even the ends of the loops deviate in the coarse spirem figure as we shall see. (Figs. D, E, F).

It will be observed that the sequence of events is at this period by no means simple, and the phases of the synapsis so far as we have now gone may be conveniently elucidated by a diagram, wherein only two double and one triple chromatin centres and their associated loops are drawn. (See next page.)

At a somewhat later stage the loops assume the arrangement given in fig. D. They each consist of a long twisted thread, all ending a few composite chromatin centres that have arisen through the amalgamation into groups of those previously scattered through the nucleus.

The thread consists of linin, along which the chromatin is scattered in the form of irregular granules, as shown in the figure. There is no trace up to this stage of any longitudinal splitting in the thread work. In fig. D, however, we have a somewhat later stage than fig. C. In this the loops are still seen to radiate from composite chromatic centres, but these are now breaking up again. And in some loops it will be seen that the chromatin is now distinctly arranged in two rows. The loops are, in fact, in the act of assuming the well-known double beaded appearance.

There is no more doubt in the case of these mammalian cells than there is in those of Triton that the double beading of the threads arises through a separation of the chromatin along each individual thread into two rows. But it is questionable whether each of the chromatin granules divides, or whether they gradually become arranged in this manner.

At a later stage (fig. E) the chromatin centres become separated up, and each is now seen to consist of the peripheral ends of the individual loops.

The double, or split, condition of the thread now extends from end to end of the loops, and this gives to each end, or chromatin centre, on the nuclear membrane, a new appearance of being double.

A later stage is represented in fig. F. The double or split nature of the chromatin is still seen. The loops are, however, shortening up to form the adult gemini.

* Moore and Embleton. On the Synapsis in Amphibia. 'Proc. R.S.,' 1906.

A

Fig. A.—Early prophase of 1st Maiotic Division from Testis of Guinea pig, showing Chromatin centres.

B

Fig. B.—Prophase of 1st Maiotic Division, showing Synaptic Contraction and Chromatin centres.

C

Fig. C.—Later stage of Synaptic figure than *B*. The loops are still unsplit.

D

Fig. D.—Later stage of Synaptic figure. The loops are beginning to show double beading, and the Chromatin centres are separating from one another on the surface of the nuclear membrane.

E

Fig. E.—Later stage, showing the formation of the coarse spireme through the complete separation of the Chromatin centres. The threadwork shows the double-beading and splitting.

F

Fig. F.—Later stage, showing the split threadwork of the loops, the split extending to their ends.

G

Fig. G.—Still later stage, showing the formation of the gemini (heterotype chromosomes) out of the split loops. Two of the gemini show the joint between the two premaiotic chromosomes of which each is formed.

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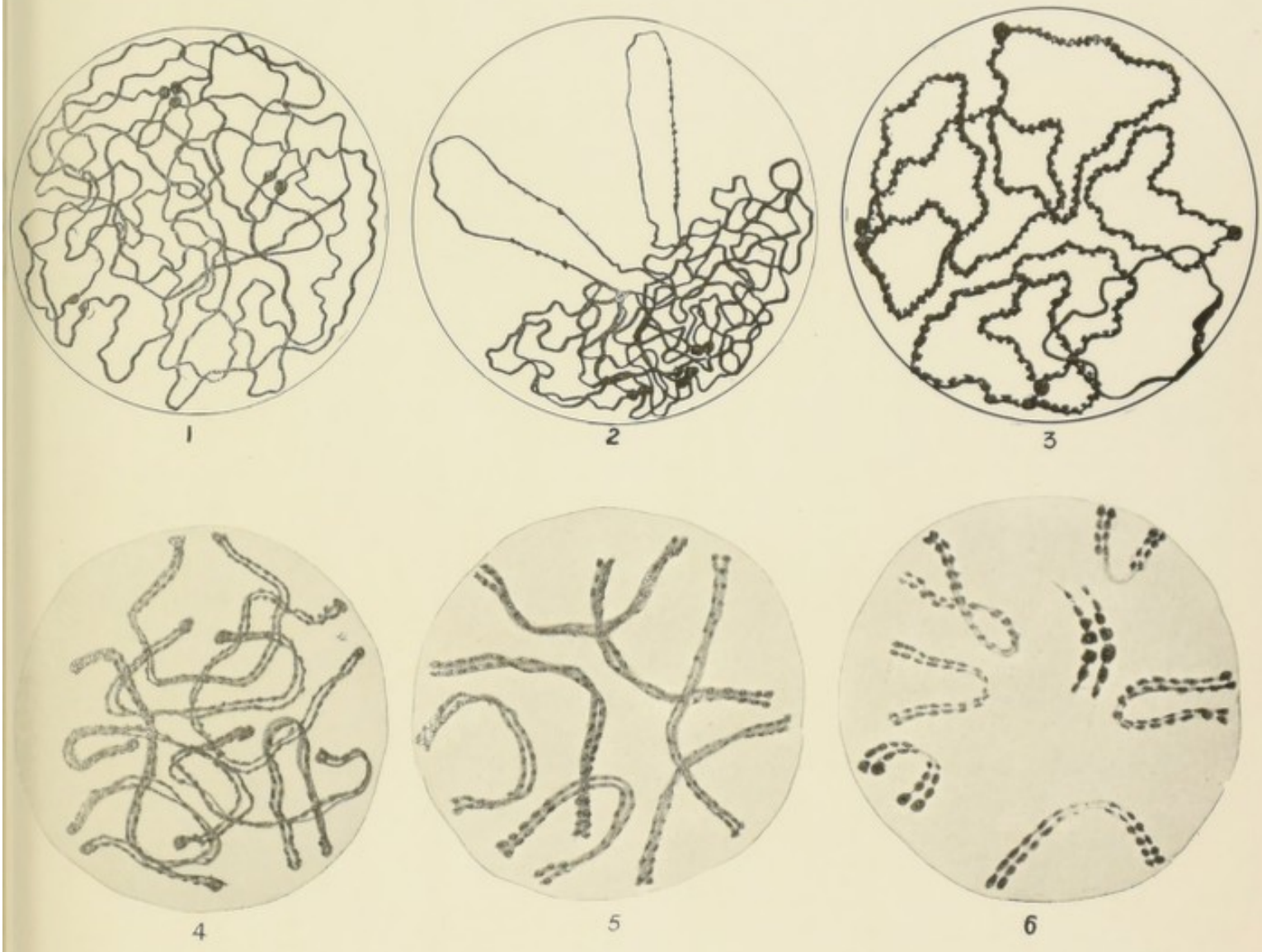


Diagram of the first maiotic prophase in the Guinea pig, wherein only seven loops are represented :—

1. Early spireme and chromatin centres at the ends of the loops. The chromatin centres are in groups of two and three.
2. Synapsis.
3. Later phase where the chromatin centres have separated and one is breaking apart on the right.
4. Later stage where the ends of the loops are all broken apart and the thread is split.
5. Still later stage.
6. —Final stage, the loops contracting to form the gemini (heterotype chromosomes). Several of the loops show the joint in their middle, representing the point of opposition of the two premaiotic chromosomes out of which each of the gemini is formed.

face p. 11

In fig. F it will be seen that these loops are often clearly divided in the middle. Thus at this stage each loop consists of two lengths joined by their ends, and each length is split longitudinally from end to end.

In the guinea-pig there are, as we have seen, 32 pre-maiotic chromosomes, and the synaptic loops of the first maiotic division resolve themselves into 16 gemini, so that we are led to conclude that gemini consists each of two somatic chromosomes joined end to end. This conception is fully confirmed by the observations we have made upon the synapsis in Triton, where the coupling up of the pre-maiotic chromosomes can actually be seen during the pre-synaptic rest.

During the time that the coarse spirem is breaking up to form the sixteen synaptic aggregates, remarkable changes go on in relation to the constituents of the attraction sphere. During the synaptic contraction it will be remembered that the centrosomes lay at the centre of an enlarged archoplasmic mass as in fig. 14. But at stages such as those represented in figs. 18, 19, 20, 21, it is found that the centrosomes migrate outwards from the archoplasmic centre to the surface of that body as in fig. 18. Still later they are encountered completely outside the archoplasm, as in fig. 20, and at this time it is often noticed that one or both of these bodies has divided, as in fig. 21. About the same time it is in some cases—in the guinea pig—possible to discern that from each of the centrosomes there proceeds a delicate protoplasmic thread of extreme tenuity, and only visible if the preservation and staining of the cell be exceptionally good. These processes seem to correspond to the rudimentary tails originally described by us* in relation to the daughter elements of the first maiotic division in elasmobranch fishes, and subsequently encountered elsewhere in corresponding cells by Meves and others. They are in all cases undoubtedly equivalent to the tail of the Spermatozoa and their appearance in the heterotype prophase of mammalia is distinctly interesting.

While the above changes affecting the centrosomes are in progress other phenomena of a no less important character make their appearance in the archoplasm itself. During the migration of the centrosomes and after, it is seen that within the substance of the archoplasm small clear vesicles appear. They are of minute size, and often in considerable numbers. In each vesicle there is usually to be seen a small dark spot, and the archoplasm presents the appearance represented in fig. 21.

Thus, so far as the attraction sphere is concerned, it may be said that the onset of the first maiotic division is marked by a peculiar migration of the centrosomes; the development of rudimentary flagellae in connection with these bodies; and the appearance of archoplasmic vesicles.

When the first maiotic division is drawing to a close, and the cells assume the hour-glass appearance represented in fig. 26, the subsequent separation of the daughter elements is marked by the formation of an intervening spindle remnant, which for a time exists as a bridge between the cells. In the centre of this structure there are to be seen one or more stained particles, which have the appearance of thickenings on the original spindle fibres, and correspond to Fleming's intermediate bodies. In all other respects the reconstruction of the daughter cells and the recession of the active nuclei into rest corresponds to what has been described in the case of the pre-maiotic divisions.

* Moore. On the Spermatogenisis in Elasmobranchs. 'Quart. Journ. Micro. Sci.,' 1895.

THE SECOND MAIOTIC (HOMOTYPE) DIVISION.

The daughter elements of the first maiotic division are represented in their resting condition in fig. 27. In all stages after their formation the archoplasm is large, while it seems doubtful in many instances if the centrosomes ever come to lie in the archoplasmic mass. At all events, these bodies in a very early stage are visibly detached from the rest of the sphere, and consequently it is obvious that in this generation there exists the same dismemberment of the constituents of the attraction sphere which marked the onset of the maiotic change. Besides the extra-archoplasmic position of the centrosomes the archoplasm itself becomes again filled with clear vesicles (figs. 27, 28); while there exists as well the conspicuous irregular chromatic body in the cytoplasm (fig. 28).

Thus, in this generation, all the peculiar cytoplasmic features which were assumed at the beginning of the maiotic change are again in evidence.

The cells produced by the heterotype division remain in a resting condition a relatively short time, and the onset of the second maiotic (homotype) division is ushered in, so far as the nucleus is concerned, by the formation of chromatic condensations, which foreshadow the chromosomes of the subsequent mitosis. These, in the guinea pig, are sixteen in number. No true spirem appears to be formed, and the best description of the process is to say that the chromatic granules become at first aggregated in clouds, and after a time into two rows in each. In this way the young chromosomes have the appearance of being longitudinally split (figs. 27, 28).

At the same time the centrosomes, usually quite near the periphery of the cell, migrate from one another to opposite ends of the cytoplasm. Radiations appear, the nuclear membrane vanishes, and the sixteen chromosomes become attached to the spindle as in an ordinary pre-maiotic division. The individual halves of each separating to form the daughter nuclei in a similar manner.

We have not hitherto been able to observe the formation of abortive flagellae in these cells, as in the corresponding generation of elasmobranchs.

At the close of the second maiotic division the archoplasm, together with its vesicle, disappears, as it does in the preceding generation, and the spindle fibres form in a marked manner once more the bridging structure between the daughter cells represented in fig. .

THE SPERMATIDS.

The reconstruction of the cells produced by the second maiotic (homotype) division, which form the final spermatids, is similar to that witnessed in the preceding generation. The centrosomes appear to be dissociated from the archoplasm, and a gradual metamorphosis is inaugurated which terminates in the formation of the mature spermatozoa.

In the present paper it is intended to confine the description of the events which lead up to the production of the mature male element to its earlier phases. In the first place, it is such phases only which appear to be of wide significance. Secondly, our account of these changes will be found in details not to agree completely with the work of Meves; but the differences are not of sufficient magnitude to require special treatment; whilst thirdly, in the work above referred to, the latter parts of the transformation have been so admirably illustrated and followed that any fresh description of them here seems quite unnecessary.

By the time the daughter elements produced by the second maiotic division have come to rest, the archoplasm is of conspicuous dimensions, and the centrosomes may be found in the cytoplasm, sometimes near the chromatic body, sometimes quite on the periphery of the cell (fig. 32). At an early stage archoplasmic vesicles appear, and these structures rapidly grow to much greater prominence than in either of the two preceding generations (fig. 35).

At the same time that the vesicles enlarge they decrease in numbers, until one or two only are left in the archoplasm, as in figs. 38, 39, 40, 41. In all cases it appears that only one is finally left, and this grows rapidly, presenting a well-marked membrane, as in (fig. 41). At the same time the central staining mass in the vesicle becomes more pronounced, and from it there grows out a more faintly staining material, which we will call the *intermediate substance* (fig. 42). About this time the archoplasmic vesicle (Plimmer's body), together with the remaining mass of the archoplasm (residual archoplasm) becomes attached to the nucleus, where the vesicle and its contents continue to enlarge (fig. 42). The clear fluid originally filling the vesicle is now gradually displaced by the expanding intermediate substance which grows out from the central staining body until it fills nearly the whole of the vesicular space (fig. 43). The clear substance is, as it were, forced down upon the nuclear membrane, and forms a clear collar in the manner represented in figs. 45, 46, 47, 48. The intermediate substance continues to grow, and finally begins to encroach into the cup-shaped space occupied by the clear substance until ultimately the latter assumes the form of a ring round the nucleus, as in figs. 48, 49, 50.

At this time the nuclear membrane gradually disappears over that part of the nucleus which is underneath the ring formed by the clear vesicular substance, and for a time the substance of the vesicle and that of the nucleus seem to be in for communication (figs. 49, 50, 51). The central staining body of the vesicle also grows, becomes half-moon shaped, and closely attached to the nuclear wall. In like manner the intermediate substance continues to grow, and follows the clear substance over the surface of the nucleus to the point at which the bulging annulus existed, the latter now gradually shrinking (figs. 51, 52, 53). From the zone round the nucleus now reached by the vesicle fine out-growth proceed in the manner described by Meves, and these ultimately form a hollow tube projecting beyond the opposite pole of the nucleus, and containing the centrosomes, and the base of the spermatic tail.

While the above changes have been going on the centrosomes have come to lie near the nucleus, opposite the vesicle, and from one of them a tail grows out as represented in figs. 48—52, both centrosomes, together with the base of the tail becoming eventually enclosed in a tube proceeding from the nucleus as above described.

For the further details of the completion of the spermatozoön the reader may be referred to the above cited work of Meves. It is sufficient for our present purpose to point out that the archoplasmic vesicle (Plimmer's body), after arising in the above manner, constitutes the anterior cap of the spermatozoön, and the archosome, as well as the tubular sheath for the tail.

REMARKS.

For the foregoing description it will be seen that :—

The maiotic phenomena in mammals, so far as the nuclear structures are concerned,

conform with the general scheme of this process given in the joint memoir by Professor Farmer and one of ourselves. We here reach, as was briefly pointed out in that publication, a similar result regarding the nature of the maiotic divisions as that obtained independently by Korschelt in the case of *Ophryotrocha,* and in the same year as ourselves by Montgomery, in amphibia. Still later similar results have been attained by Strasburger and others in relation to plants.

In the first maiotic (heterotype) division the synaptic aggregates are formed by the association of pre-maiotic chromosomes, and those in the ensuing mitosis simply separate from one another, the existing longitudinal split in each chromosome taking no part in the division, and persisting in the diastral stage of the mitotic figure.

The reduction of the number of chromosomes in the second maiotic figure is brought about by this peculiar mode of association and separation.

From the first inception of the maiotic change peculiarities are seen in relation to the attraction sphere. Archoplasmic vesicles appear. The centrosomes take up an extra archoplasmic position, and may acquire, apparently at any time, rudimentary tails. These observations, together with those of Meves and others, strongly confirm the position taken up by one of us in 1895 (after the discovery of the rudimentary tail in the daughters of the heterotype in elasmobranchs), that from the first appearance of the chain of maiotic phenomena the cells in all the succeeding generations in animals tend to revert, sometimes actually, and probably always potentially, to the condition of flagellate gametes.

DESCRIPTION OF FIGURES.

Unless otherwise stated the figures are made from the cells of the testis of the guinea-pig, and have been drawn under a 2mm. 1.40 n.a Zeiss Apochrom. 10-inch tube, 18oc.

PLATE I.

Fig. 1.—Resting pre-maiotic cell.
Fig. 2.—Early spirem.
Fig. 3.—Late spirem, the thread has broken up into the pre-maiotic chromosomes.
Fig. 4.—Pre-maiotic spindle figure.
Fig. 5.—Diaster.
Fig. 6.—Later stage of same.
Fig. 7.—Later stage of same.
Fig. 8.—Reconstruction, daughter nucleus returning to rest.
Fig. 9.—Resting pre-maiotic cell, similar to fig. 1.

PLATE II.

Fig. 10.—Early prophase of the first maiotic divisions. The nucleus shows chromatic centres.
Fig. 11.—Synaptic contraction.
Fig. 12.—Another view.
Fig. 13.—Another view.
Fig. 14.—Later stage of the synapsis.
Fig. 15.—Stage at which the first splitting of the thread is visible.
Fig. 16.—The contraction opening out to form the coarse spirem figure.
Fig. 17.—A later stage of same.
Fig. 18.—Later stage, the loops contracting up to form the gemini.

PLATE III.

Fig. 19.—A similar stage to 18.
Fig. 20.—A similar stage.
Fig. 21.—Later prophase showing the peculiar appearance of the young gemini.
Fig. 22.—Early spindle figure of the first maiotic division.
Fig. 23.—The same later.
Fig. 24.—The same stage in a mouse.
Fig. 25.—Diaster.
Fig. 26.—Reconstruction nuclei in daughter cells.

PLATE IV.

Fig. 27.—Resting cell before the prophase of the second maiotic division.
Fig. 28.—Prophase of the second maiotic division.
Fig. 29.—Spindle figure of same.
Fig. 30.—Diaster of same.
Fig. 31.—Daughter cells of the first maiotic division.
Fig. 32.—Resting spermatid.
Fig. 33.—Three spermatic nuclei in a single cytoplasm.
Fig. 34.—Three spermatic nuclei in a single cytoplasm.

PLATE V.

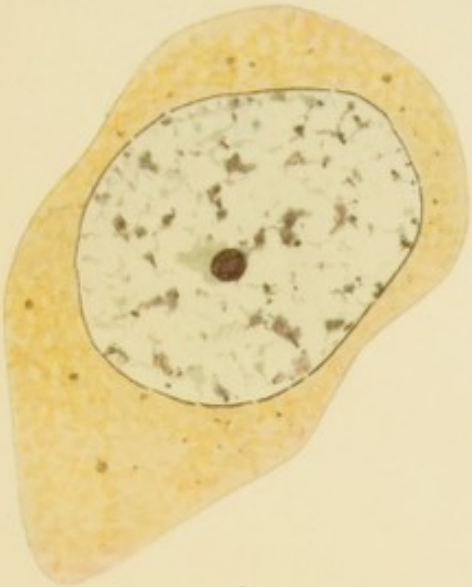
Fig. 35.—Resting spermatids, with remains of spindle between them, and first indications of the archoplasmic vesicles in the archoplasms.
Figs. 36, 37, 38, 39, 40, 41, 42.—Stages showing development of the archoplasmic vesicle.

PLATE VI.

Figs. 43, 44, 45, 46, 47, 48, 49, 50, 51.—Stages in the development of the archoplasmic vesicle.

PLATE VII.

Figs. 52, 53, 54.—Stages in the development of the archoplasmic vesicle.
Figs. 55, 56, 57, 58.—Diagrams of spermatogenesis (see text, p. 7).



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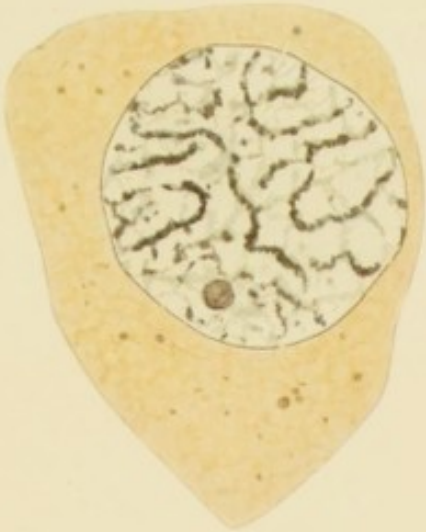

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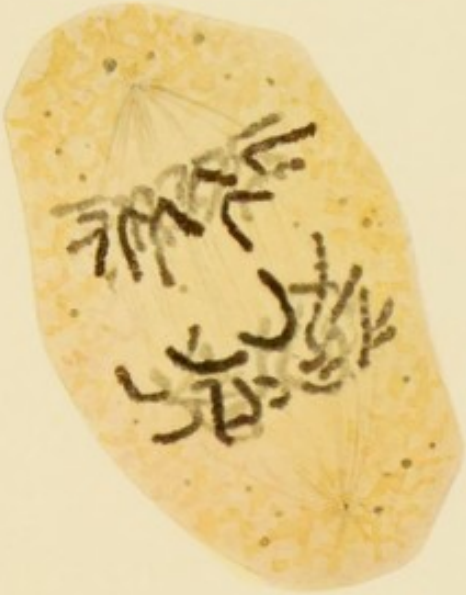

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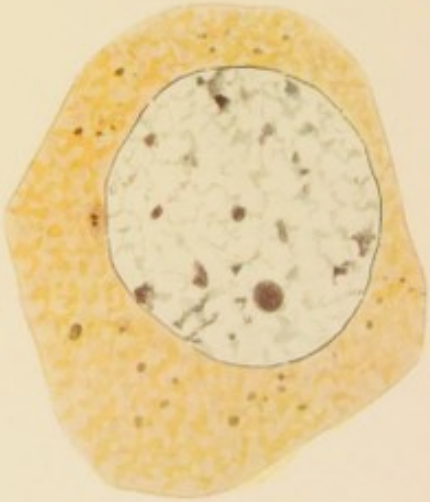

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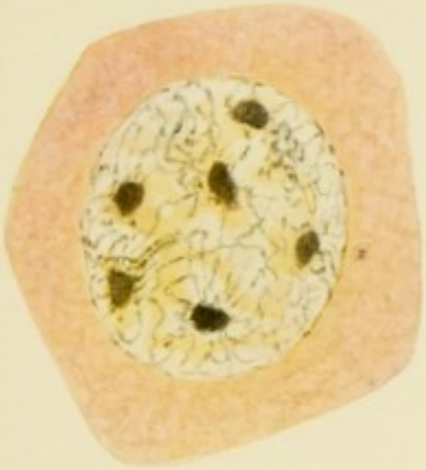

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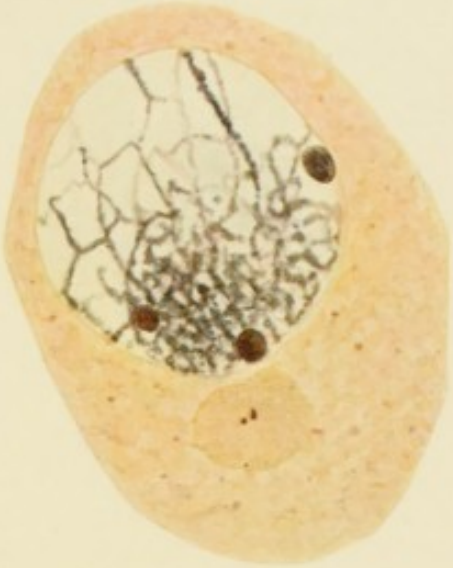

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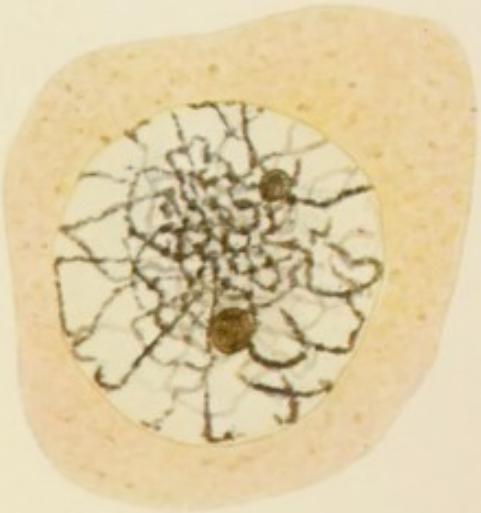

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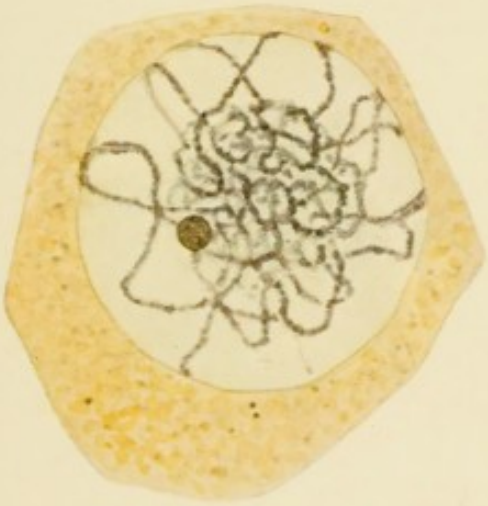

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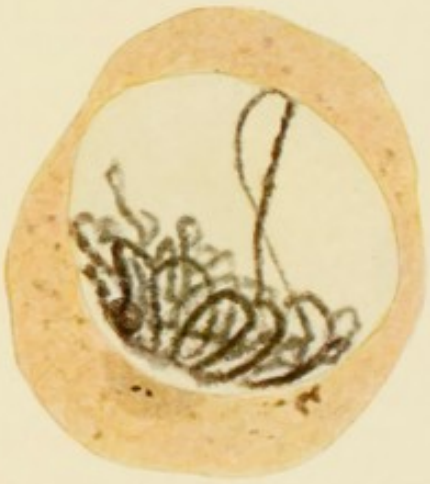

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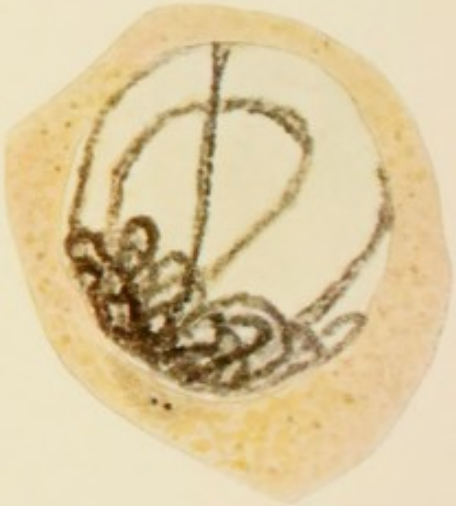

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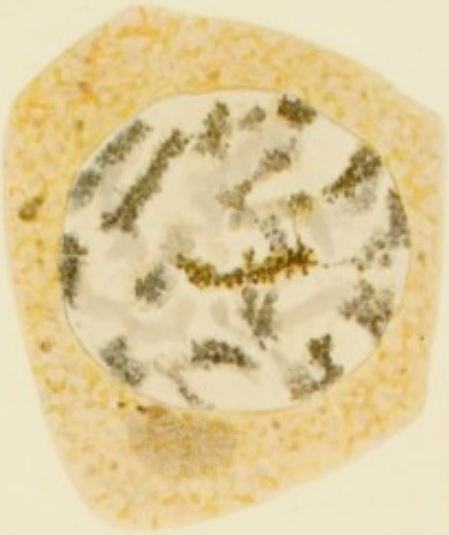

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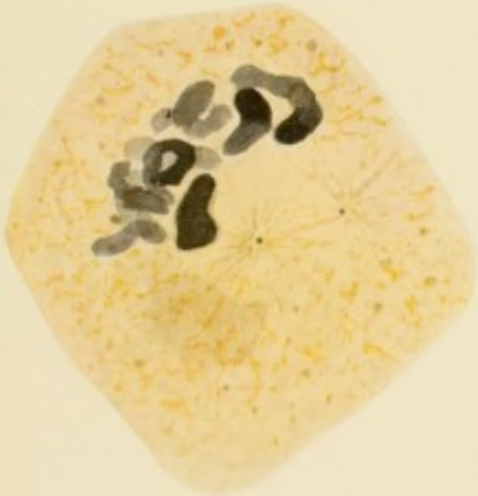

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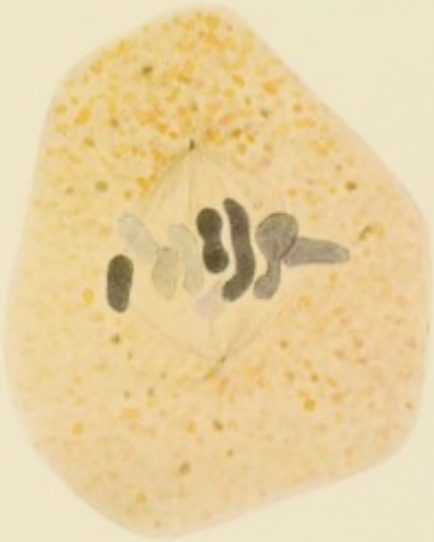

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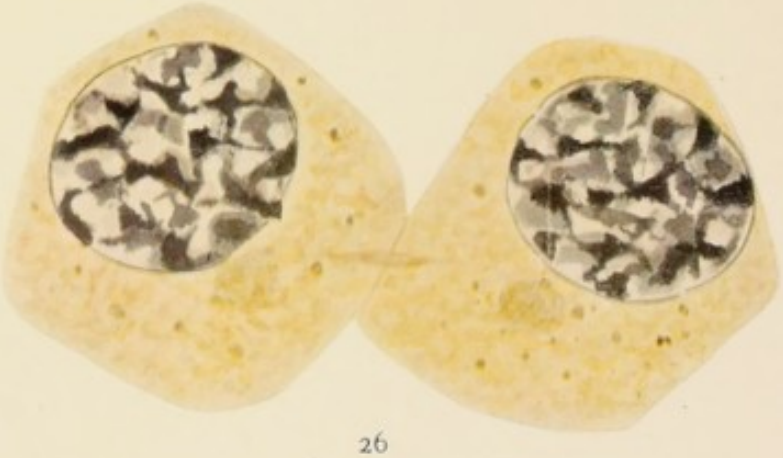

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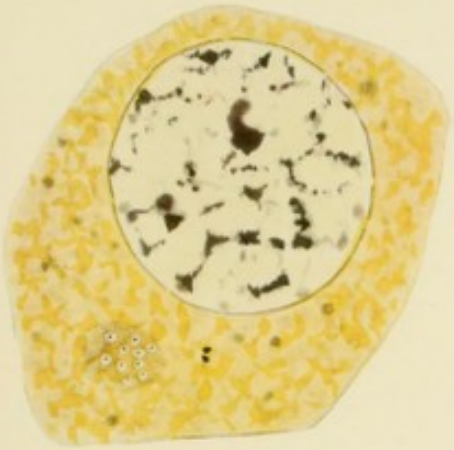

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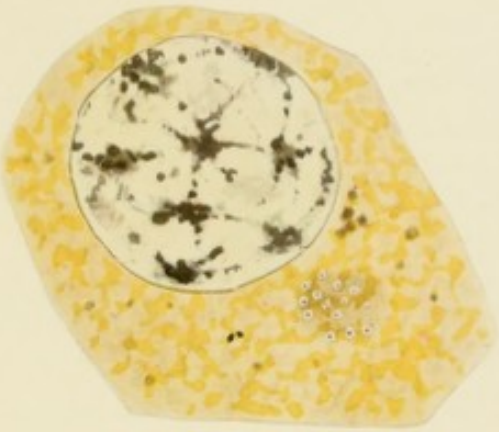

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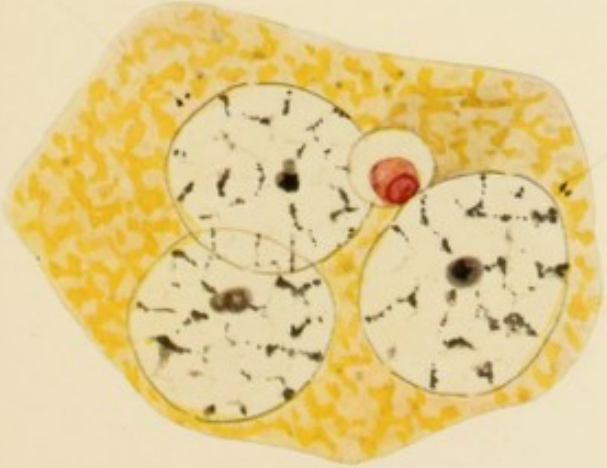

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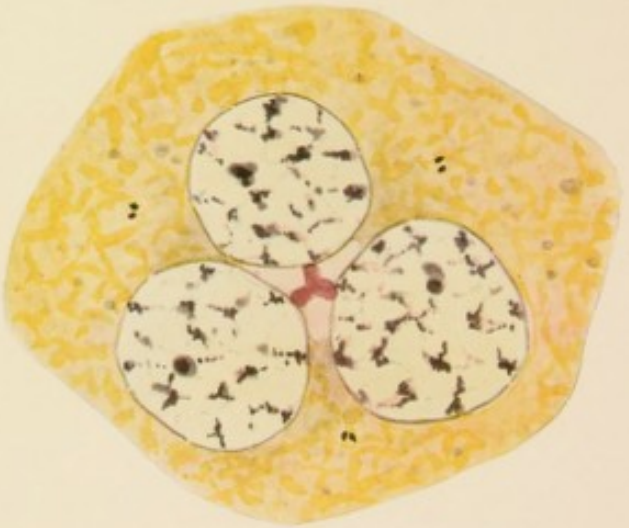

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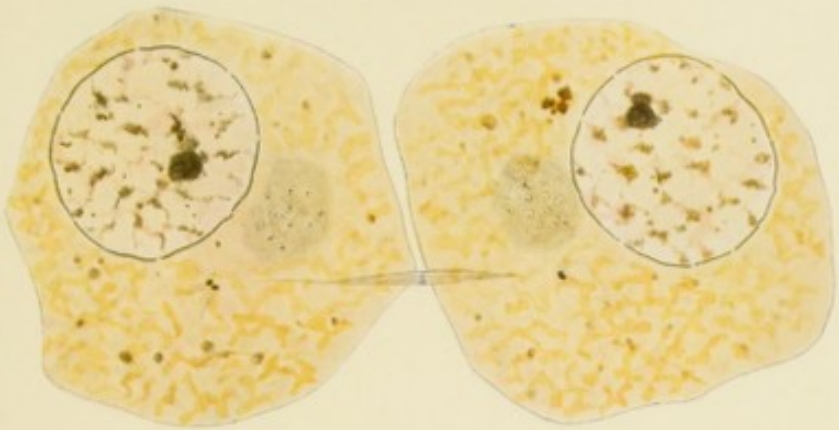

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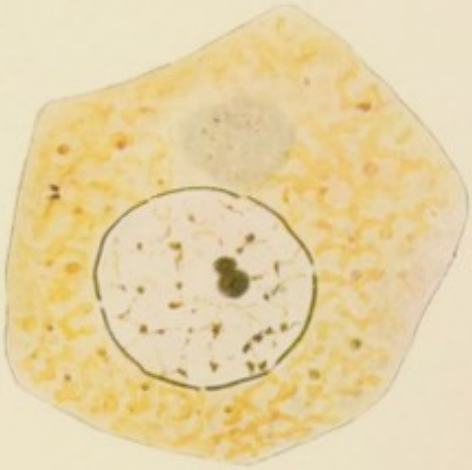

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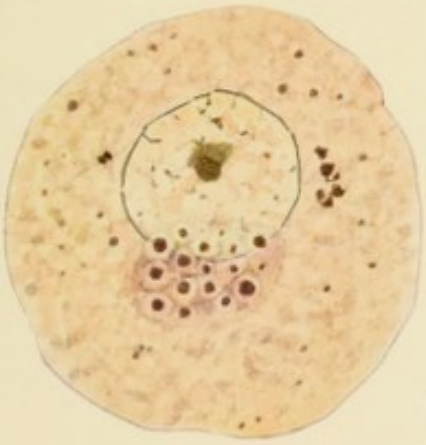

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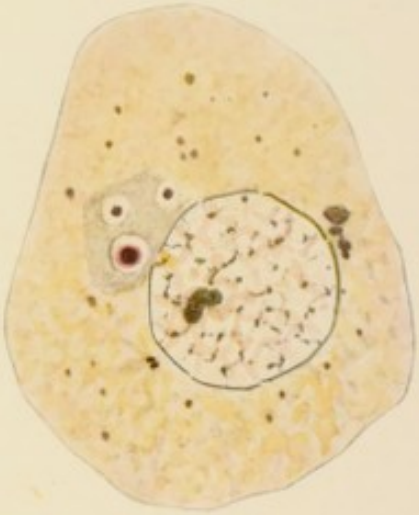

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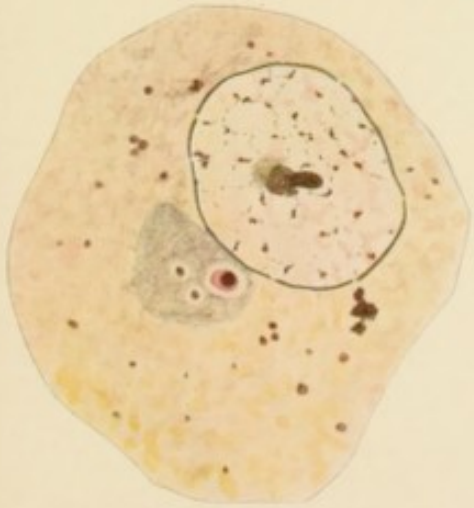

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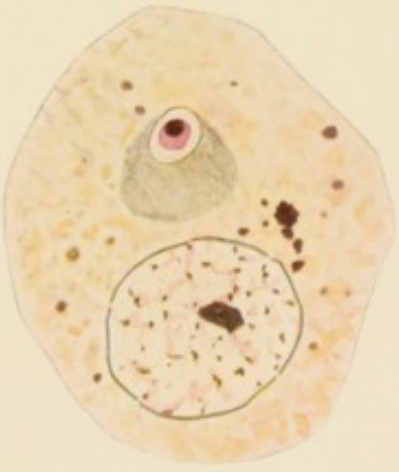

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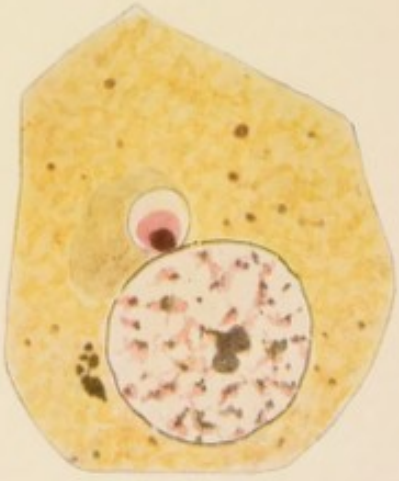

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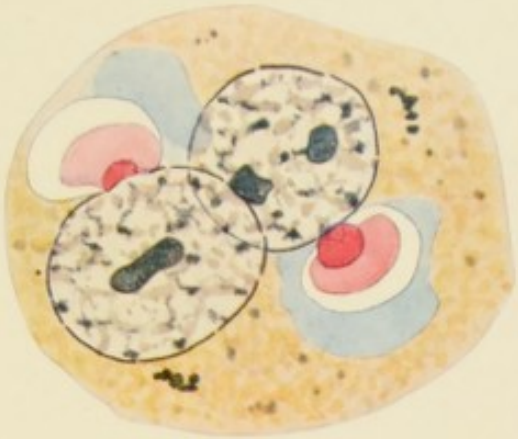

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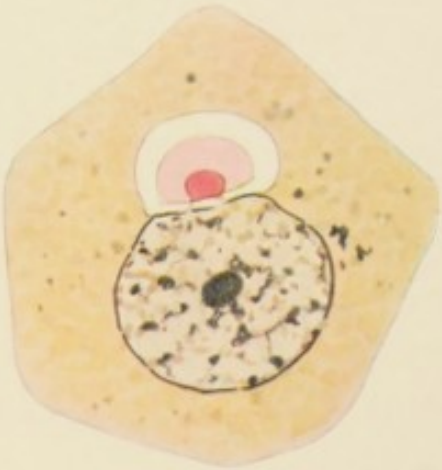

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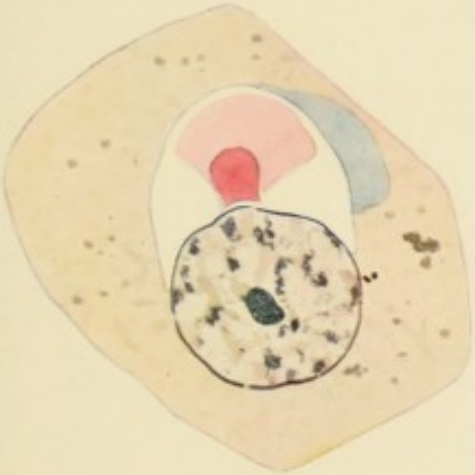

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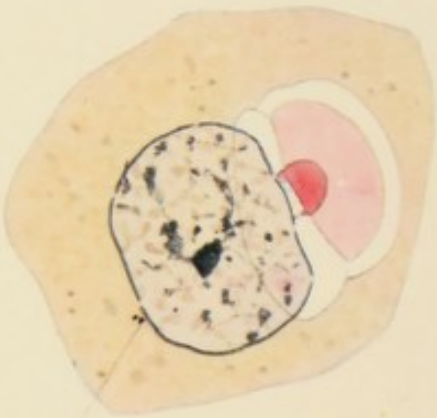

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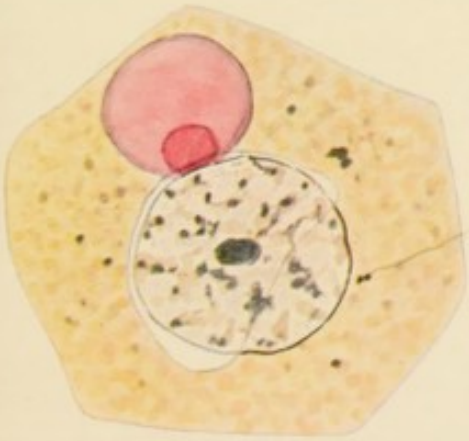

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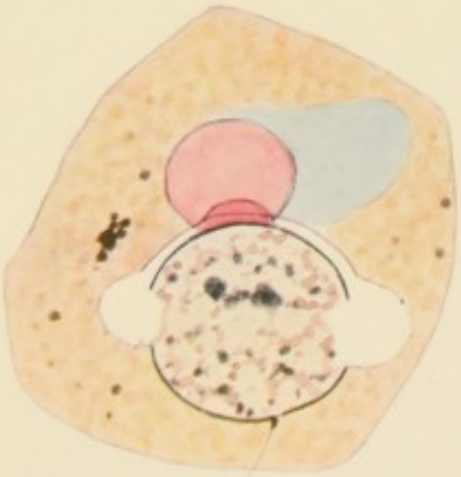

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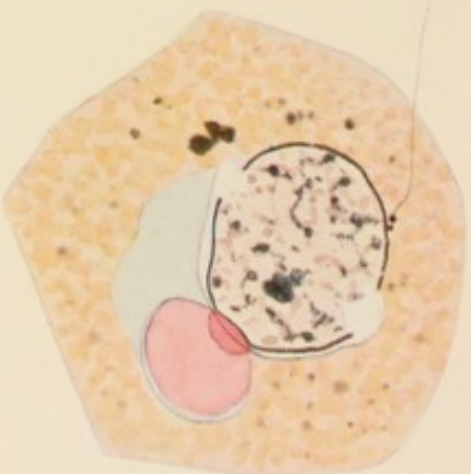

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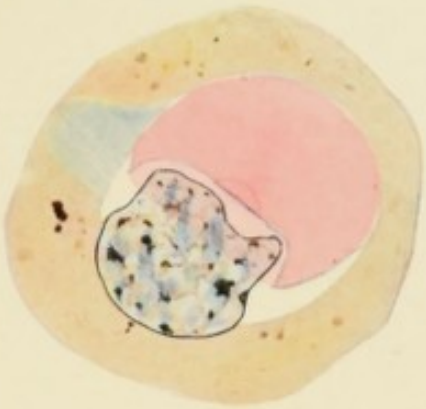

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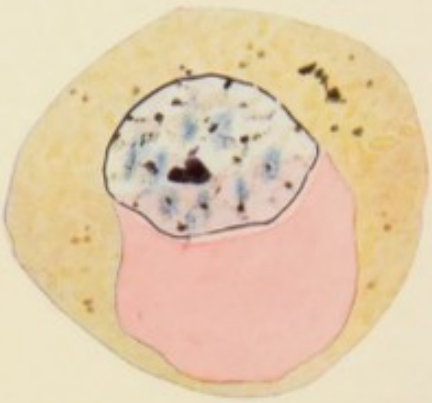

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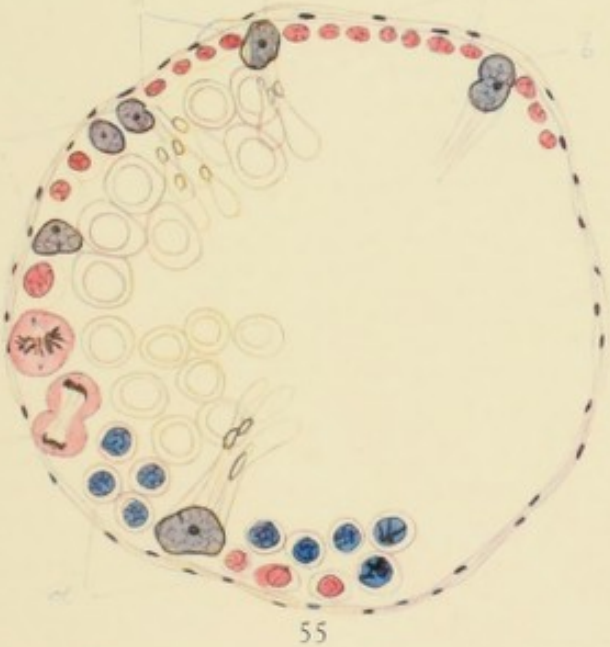

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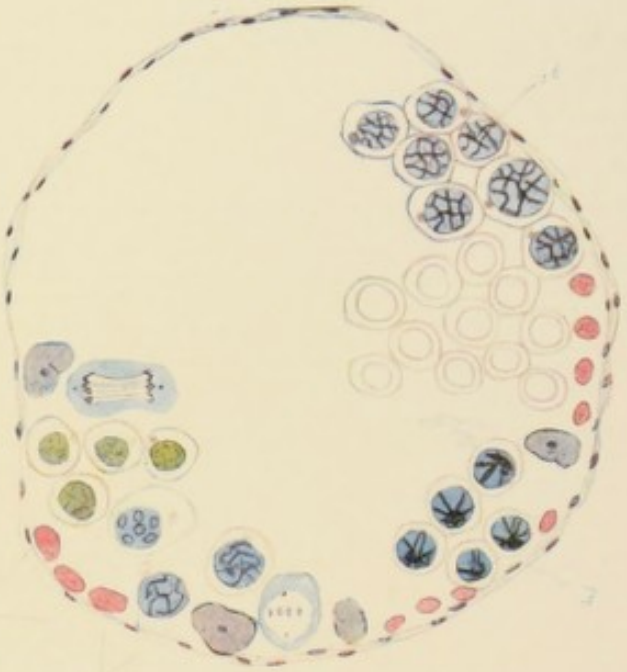

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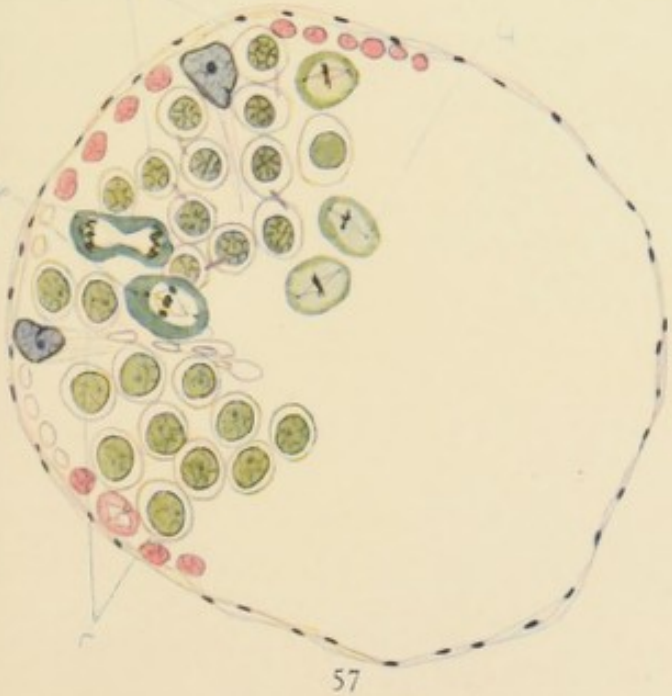

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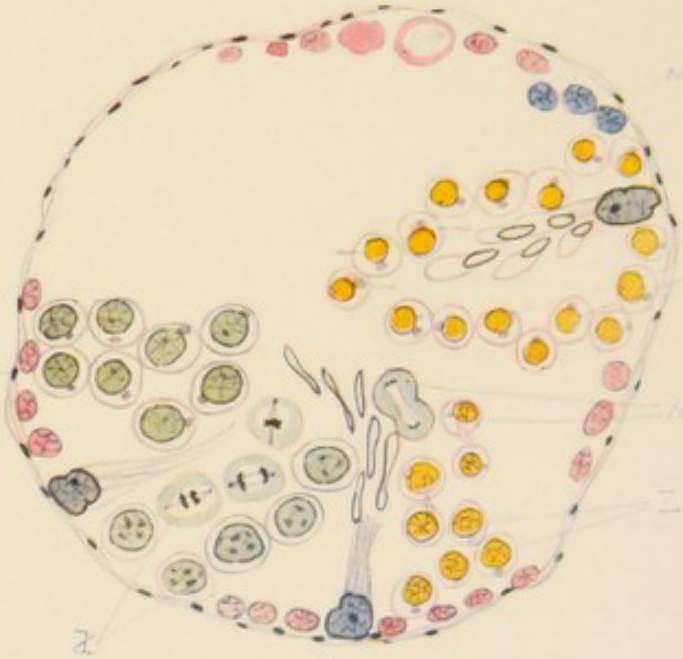

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6—ON THE SYNAPSIS IN AMPHIBIA.

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In 1903 and 1904 one of us, in conjunction with Professor Farmer, described the maiotic process in a variety of animals and plants.* From the observations then accumulated it was in the first place shown that what we termed the maiotic process appears to be the same throughout the animal and vegetable kingdoms. In the second it was pointed out that the general scheme we were able to formulate was in accord with the particular description of the metamorphosis given by Korschelt† for Ophryotrocha as long ago as 1895, as well as with the account of the same change in some amphibia given by Montgomery‡ in the same year as ourselves.

According to this conception of the maturation process, the reduction in the number of chromosomes to one half is brought about by pairing of somatic chromosomes which takes place in the prophase of the first maiotic (hetherotype) divisions. In this way we have in some mammals, for example, 16 pairs of chromosomes in the place of 32 single elements.

The chromatic *gemini,* as we propose to call them, go on to the spindle in the same way as ordinary pre-maiotic or somatic chromosomes. But during the division each of the respective gemini separate into the two component parts; so that in the cases of mammals above referred to there are 16 pre-maiotic chromosomes distributed to each daughter cell.

According to this view it would appear that during the first maiotic division no longitudinal fission of the chromosomes composing the gemini comes into play, and the longitudinal split which is visible in the spirem figure only effects that incomplete fission of the daughter elements first observed by Flemming in the diasters of the first maiotic division in amphibia (see fig. 22).

In this way the longitudinal split of the thread which takes place in the spirem stage only becomes completed and effective during the second maiotic (homotype) division. Consequently these two divisions with their respective prophases form a stage in the cell cycle of plants and animals which differs entirely from the divisional sequence before and after it.

The present communication deals with the prophase of the first maiotic division in *Triton*, and with the subsequent division up to the appearance of the diaster. And as we have worked out the stages in this animal in great detail, we propose to give the results of our observations without discussion in the text, but to indicate by means of footnotes those points in which the fresh observations differ from the existing accounts of the maturation process in general, and of the same phenomena among amphibia in particular.

It may, however, be pointed out that the results of this investigation have been entirely to confirm the general interpretation of the maiotic metamorphosis already published by Professor Farmer and one of ourselves.§

At the same time it should be noted from the beginning that this view of the maiotic process entails a complete revision of the older conceptions of the nature of the first maiotic

* *Cf.* Farmer and Moore, 'Proc. R.S.,' May, 1903; Farmer and Moore, 'Quart. Jour. Micro. Sci.,' vol. 48; Farmer and Shove, 'Quart. Jour. Micro. Sci.,' vol. 48; Moore and Robinson, 'Quart. Jour. Micro. Sci.,' vol. 48.

† Korschelt, 'Zeitschr. für Wiss. Zool.,' vol. 9.

‡ Montgomery, 'Biol. Bull.,' vol. 4, 1903.

§ Farmer and Moore, *loc. cit.*

(heterotype) division among the higher vertebrata, such as those embodied in the able works of Flemming, Meves, and many others.

During the early summer (June) in England the testis in *Triton* are found to present all the phases in the cell cycle, from pre-maiotic cells dividing in their characteristic manner, to the spermatids that have been produced by the second maiotic (homotype) division (see fig 1, Plate).

From this figure it will be seen that in the section of the testis which the drawing represents the spermatogenesis is proceeding from the top towards the bottom of the Plate. Immediately below the peritoneal attachments groups of cells are seen, some with their nuclei at rest, others dividing; while in the region marked *c* nuclei are seen as we pass downwards, which are at first indistinguishable from those in *b*, but which gradually alter in the appearance until we reach the lower part of *c*.

The division figures encountered in region *b* are all of the ordinary pre-maiotic type, and the appearance which comes over the resting nuclei in *c* as we pass downwards is the appearance produced by the advent of the synaptic metamorphosis in *Triton* when seen under a low power. Still lower than the region *c* the two maiotic divisions (heterotype and homotype) are encountered together.

The above will serve to orientate the original and relative positions of the cells which have been taken as individual illustrations of the successive phases of the synaptic change. But before leaving the small scale figure, it may be noted that upon examination the resting nuclei in region *b* differ from those in the *c* area in that they obviously contain more of the apparently nucleola bodies scattered within the membrane.

When resting cells of the region *b* are examined under a high power, it is seen that nucleolus-like bodies visible under a lower power resolve themselves into the short chromatic rods represented in figs. 2 and 3.

These bodies are seen to lie more or less parellel to one another within the cells, so that the appearance of the nuclei varies from that given in fig. 2, where the chromatic rods are seen from one side, to that represented in fig. 3, where the same rods are looked at from one end.

The definite nature of these bodies is sufficiently obvious from the figures, but their significance only became apparent upon ascertaining that their number is always about 24; that is to say, it was found, after counting in about 50 individual cells, that the number 24 was obtained for the great majority, while in the few cases in which it was above or below this figure such divergence was limited to one or two, and in nearly all these exceptions it was possible to attribute the divergence to some optical difficulty. The bodies lay over one another in the line of vision, or were hidden and confused by the nuclear membrane and the like.

The pre-maiotic resting cells in the testis of *Triton* are found then to contain in their nuclei chromatic structures which at first might be, and generally have been, regarded as "chromatin nucleoli" (Flemming); but they correspond in numbers to the chromosomes of the pre-maiotic prophases and division figures, for in this animal the number of the pre-maiotic chromosomes is 24. These bodies correspond in fact exactly to what in 1904 we have already described as the Anlagen of the pre-maiotic chromosomes in the corresponding cells in the testis of *Periplaneta*,* and there can be no doubt that they represent also the structures subsequently alluded to as prochromosomes by Overton,† Miyake,‡ and Strasburger§ in the same stage in certain mono- and dicotyledonous plants.‖

In *Triton* these structures are not restricted to the pre-maiotic cells of the testis, but are equally apparent and have the same relationships in other tissues of the animal's body.

In some respects the bodies in question are more definite and stainable in the resting pre-maiotic cells of *Triton* than they are in the corresponding elements of *Periplaneta*, and in both cases their subsequent history during the pre-maiotic divisions is perfectly simple. In *Triton* each Anlagen (or prochromosome) becomes gradually enlarged and thickened into the long pre-maiotic chromosomes of the spirem figure, while in *Periplaneta* they gradually assume the form of dense short rods characteristic of the pre-maiotic division figures of that arthroped.

In both cases the chief interest of these bodies lies in the fact that they obviously represent the chromosomes of division during rest; and we may say without reserve that their presence at all stages of rest between the successive pre-maiotic divisions seems to conclusively prove the permanence of the chromosomes from one cell generation to another.

Examination of the resting maiotic nuclei given in figs. 1 and 2 shows that, so far as can be seen, the Anlagen consist of irregular rods composed chiefly of chromatin, and either suspended in a fine linen meshwork within the nucleus or attached to the nuclear membrane, where the chromatin is seen to be spread out, and to give to the membrane itself the peculiar thickened appearance characteristic of the stage. No other structures with the exception of irregular nucleoli are visible within the resting pre-maiotic nuclei.

Before the maiotic metamorphosis sets in the Anlagen are seen to be single and discrete, figs. 2, 3; but as we pass to an examination of the cells in the later phases in the region *c*, where the synaptic change is gradually proceeding towards the formation of the coarse spirem, we find that the Anlagen are no longer single, in fact, the diminution in the number of what, under a low power would be taken for nucleoli is seen to be produced by a pairing of the bodies while the nuclei themselves still remain at rest.

The appearance which such cells present is represented in figs. 1, 4, and 5. Fig. 4 shows a cell in this condition in the same relative position as that represented in fig. 2. The associations produced in this way (which we propose to call *gemini*) are again represented in fig. 5, where the gemini are seen end on, as were the single Anlagen in fig. 3.

We have then in the testis of *Triton* pre-maiotic cells which, like the pre-maiotic or somatic cells of rest of the animal's body, possess structures that represent during rest the individual chromosomes of the successive division figures, and these bodies during the first phase of the synaptic metamorphosis that is perceptible, conjugate or pair so as to form 12 gemini in the place of the 24 single Anlagen (prochromosomes).

When the gemini have been produced it is seen, as in fig. 4, that they are related in a conspicuous manner by shreds and strings of linin to chromatin patches on the membrane of the nucleus; and there are indications that before the gemini are formed the linin of each single chromosome extends out to the limits of the nucleus.

At all events, when the gemini have been produced each chromatic rod rapidly extends outwards along linin threads in the manner represented by fig. 6. In this we soon get an appearance which would be quite easy to misinterpret as a fission in a spirem were not the earlier stages of the process present in the surrounding tissue.

* Farmer and Moore, 'Quart. Journ. Micro. Sci.,' vol. 48, *loc. cit.* Plate 38 and text.
† Overton, J. B., 'Über Reduktionsteilung in dem Pollenmutterzellen einiger Dikotylen.'
‡ Miyake, K., 'Über Reduktionsteilung in dem Pollenmutterzellen einiger Monokotylen.'
§ Strasburger, E., 'Typische und Allotypische Kernteilung.'
|| 'Jahrb. für Wiss. Botanik,' vol. 42.

A somewhat later stage is represented in fig. 7, where it is seen that the original components of the gemini are rapidly elongating out into loops or polarised chromatic bands. Cells possessing the characteristics represented in figs. 6 and 7, constitute, in fact, the only representative in *Triton* of the strong synaptic contraction figure so conspicuous in many other forms of animals and plants. At a later stage the gemini, although still clearly visible, have so far elongated and moved from their original positions as to give the appearance represented in fig. 9.

This movement, which corresponds to the unwinding of the synaptic loops in mammals and other forms, is seen to have reached a further stage in fig. 8. Here the condition of the early coarse spirem stage is clearly foreshadowed, and by stages such as those represented in figs. 10 and 11 the characteristic polarised loops of the late spirem figure become gradually formed.

It appears then that the polarised loops are produced by the growth and elongation of the original gemini, and that consequently each loop represents two pre-maiotic chromosomes, which may be associated together at one, or both, of their ends. In general, in *Triton* and elsewhere the chromosomes forming the loops remain connected together only by the ends which originally lay towards the nuclear interior, the outer pair of ends abutting upon some portion of the nuclear membrane, and always in the late stages of the spirem figure becoming widely detached from each other.

Stages in the formation of the individual loops and the mode of attachment of the chromosomes together at the round ends of the loops can be seen in figs. 9, 10, 11, and 12.

From the original formation of the gemini until the production of the spirem loops, figs. 10 and 11, the chromatin in each lateral component or chromosome is seen to be in the form of irregular granules, and remains scattered along a linin framework; but at subsequent stages (figs. 12, 13, and 14) these chromatin particles become arranged, or split as it were, into two longitudinal rows, and the loops from this time onwards present the characteristic split appearance represented in figs. 13, 14, 15, and 16. In stages such as that given in fig. 14 every loop is completely divided throughout its entire length, and the longitudinal halves of the thread may divaricate from each other as far as is to be seen in portions of the loops represented in fig. 15. At this stage, and after, the loops are very long indeed, stretching in some cases completely round the whole nucleus, and it is, we think, without doubt chiefly owing to this circumstance that their history has been in general misinterpreted.*

A comparison of figs. 14, 15, and 16 will show that the split after becoming, as in fig. 15, very conspicuous, gradually closes up again, fig. 16, whilst in figs. 17 and 18 the closing process is still further completed. Even in fig. 18, however, the split is in places still visible,

* As is well known, Flemming, and after him many others, originally regarded the split of the spirem as opening out in lengths to form the rings and loops presented by the adult so-called heterotype chromosomes, and the longitudinal fissions of the daughter elements in the diaster of this division as a subsequent and independent fission. Dixon ('Roy. Ir. Acad. Proc.,' vol. 3) regarded the split seen in the spirem as due to an approximation similar to that witnessed in the formation of the gemini. Berg ('La Cellule,' vol. 21), Overton, and Miyake (*loc, cit.*) have adopted a similar view, regarding the late split in the spirem as a lingering expression of the conjugation during the formation of the several gemini. At times a similar view has been taken by Strasburger (*loc. cit.*) and others. We regard our present observations, as well as those upon numerous other forms dealt with in our former paper with Professor Farmer, as incompatible with the ideas contained in the works of the above authors, namely, that the split in the spirem of the first maiotic division is due to an approximation, and are inclined to think that this view can only have originated through a confusion having been made between the conjugation during the formation of the gemini and the longitudinal fission which, without any doubt whatever, does take place during the spirem stage.

Montgomery ('Biol. Bull.,' vol. 4), in the same year as ourselves (Farmer and Moore, 'Roy. Soc. Proc.,' 1903, *loc. cit.*), regarded the split in the spirem in Amphibian heterotype prophases as not constituting the opening of the loop or ring of the spindle figure, and the fission of the daughter diastral elements as due to the split visible in the spirem.

and at fig. 19, when the loops have been finally resolved into the adult gemini of the first maiotic division figure, the fission is not in all cases completely lost.

In other animals at the same stage, *Periplaneta* for example, the fission, as has been shown in a former work,* is often visible throughout the whole spindle figure of the first maiotic mitosis, and in such cases the origin of the fission of the daughter chromosomes is obvious.

In the final stages of the prophase in *Triton* (figs. 18 and 19) the gemini assume the various forms characteristic of the first maiotic division, and during the diaster the rings and loops break apart in the manner represented in figs. 21 and 22.

As soon as the diastral V's have been formed the original longitudinal split becomes here also again clearly visible (fig. 22). It is moreover quite easy to show in *Triton* as in other cases that it is this fission which functions in the final maiotic (homotype) division.

To recapitulate (see diagram):—In *Triton* it is found when sufficient optical power and

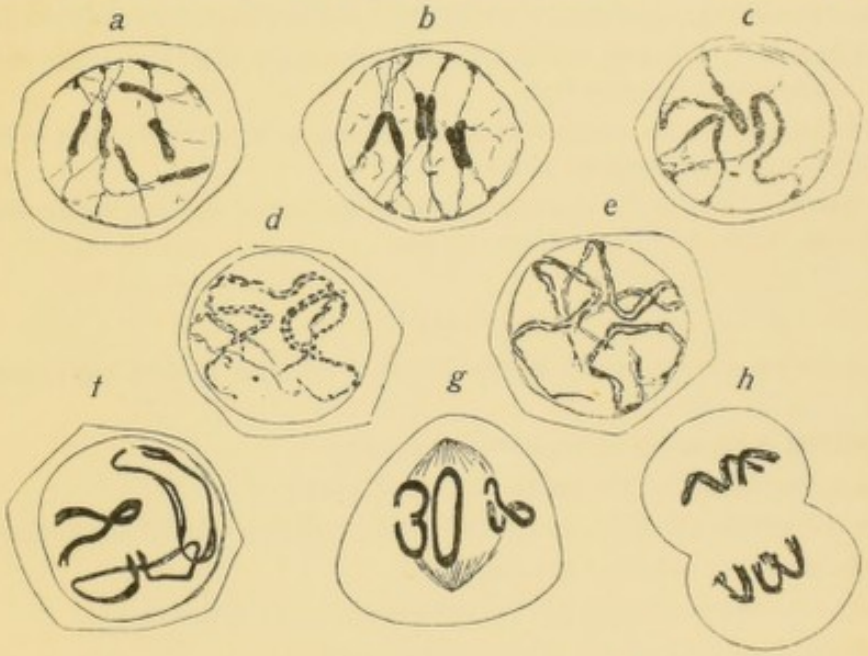

Diagram representing the course of the synaptic metamorphosis. For the sake of clearness only six chromosomes are represented in the pre-maiotic resting cell *a*. At *b* the chromosomes are uniting; *c* shows the conversion of the gemini into the young loops. At *d* is seen the split in the chromosomes; *e* shows a later stage; *f* shows the transformation of the loops into the adult gemini (heterotype chromosomes); *g* represents their appearance on the spindle; *h* shows the diastral chromosomes, three in each cell, with the longitudinal split begun in the preceding prophase.

efficient preservation are combined, that: The somatic chromosomes are visible in the resting cells; that during the inception of the synaptic phase these chromosomes pair so as to form double bodies which are the forerunners of the adult gemini (heterotype chromosomes, allotype chromosomes, bivalent chromosomes, etc.); that by growth and elongation the gemini constitute the polarised loops of the first maiotic prophase; that these loops become longitudinally split, and later each longitudinally fissed aggregate rolls itself up into one or other of the forms assumed by the adult gemini; that in these later stages in *Triton* the longitudinal fission of the chromosomes becomes almost, but not quite, closed up, and in the diaster the separated chromasomes again exhibit it; while finally it is seen that this split functions in the second maiotic (homotype) division.

* Farmer and Moore, 'Quart. Journ. Micro. Sci.,' vol. 48, *loc. cit.*

DESCRIPTION OF PLATES.

[Figs. 2—22 are drawn with a 3-mm. long-tube Zeiss apochromatic 1·40 aperture, and a 27 ocular.]

Plate I.

Fig. 1.—Section of Testis of *Triton* (June) as seen under a low power lens. At the upper margin is the attachment between the testis and the body wall and the membrane which surrounds the tubules. The foot-cells are not shown. Near the upper margin to the right are two "male ova"; towards the left are tubules with cells in the pre-maiotic (somatic) stage dividing at *b*. The region marked *b* contains the zone of transformation wherein the synaptic gemini are constituted. Late synaptic (heterotype) prophases are seen at *c*, and the first and second maiotic divisions lower down.

Fig. 2.—Cell from the upper pre-maiotic region, the nucleus showing bodies representing the 24 chromosomes while in a condition of complete rest.

Fig. 3.—Cell from the same region as fig. 2, viewed from a direction at right angles to the foregoing; the bodies representing chromosomes seen end on.

Fig. 4.—Cell in the first phase of the synapsis, showing the bodies representing the resting pre-maiotic chromosomes uniting in pairs to form the gemini.

Plate II.

Fig. 5.—Cell from the same region as fig. 4, viewed from a direction at right angles showing the gemini end on.

Fig. 6.—Cell advancing in the synapsis, the gemini elongating.

Fig. 7.—Synapsis further advanced, the gemini elongating into loops.

Fig. 8.—Synapsis still further advanced, the gemini moving from their original central position.

Fig. 9.—The gemini becoming converted into loops.

Fig. 10.—The gemini becoming polarised into the loops of the coarse spirem figure.

Fig. 11.—Cell showing the coarse spirem, and threads not split.

Fig. 12.—Same as fig, 11, but showing the first traces of the longitudinal split.

Figs. 13, 14.—Longitudinal split more apparent.

Plate III.

Fig. 15.—The split seen at its maximum.

Fig. 16.—The split seen closing up at places.

Figs. 17, 18.—Cells showing the ends of the loops with portions of the split closed up, others open.

Figs. 19, 20, 21.—Stages in the first maiotic division.

Fig. 22.—The diaster of the first maiotic division, showing the split in the diastral chromosomes.

PLATE I.

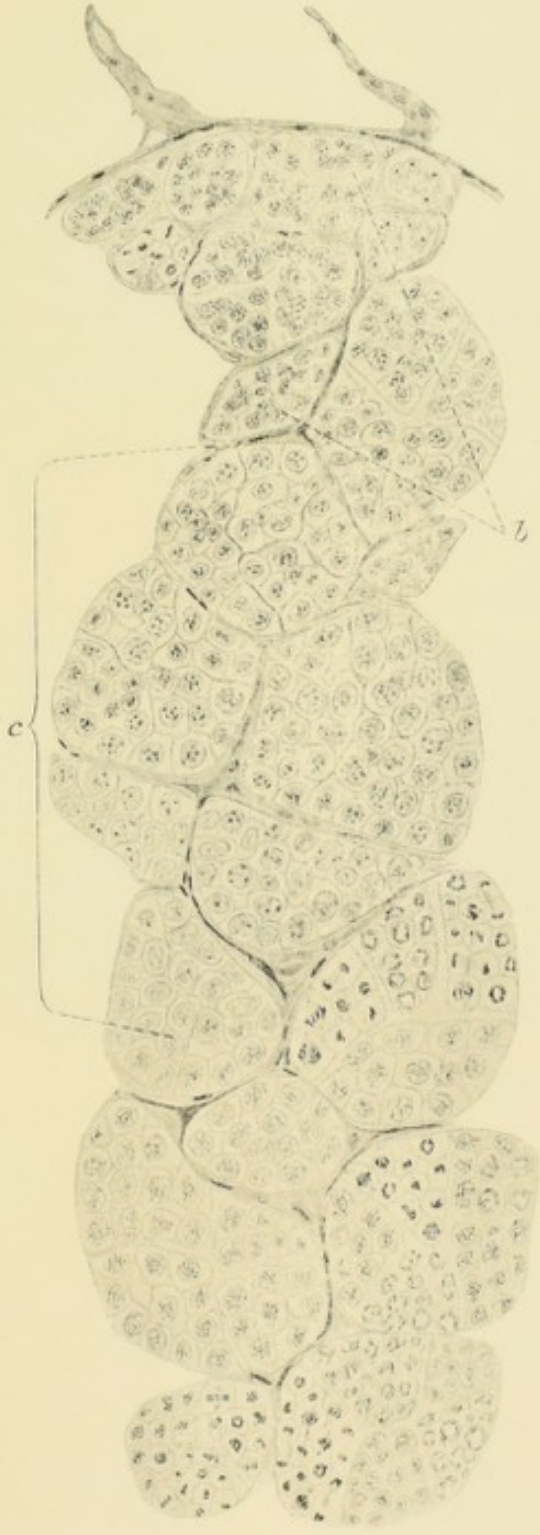

Fig. 1

Fig. 2

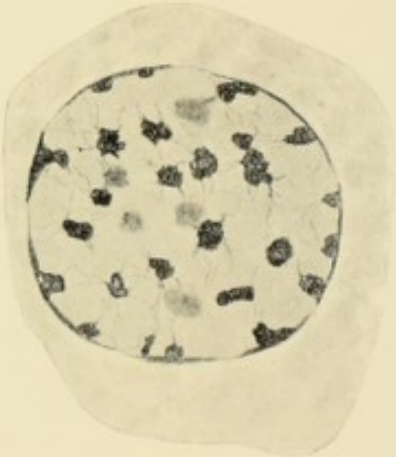

Fig. 3

Fig. 4

To face page 72

PLATE II.

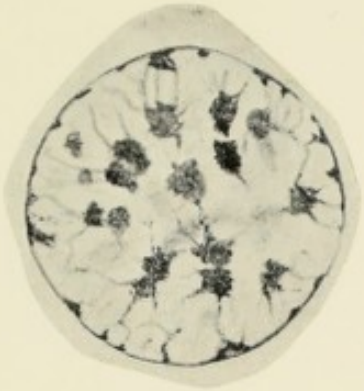

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

PLATE III.

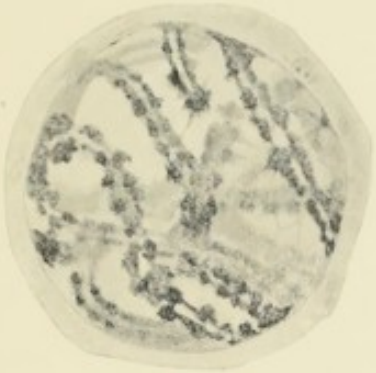

Fig. 14

Fig. 15

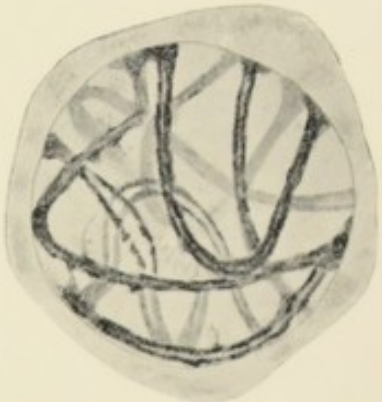

Fig. 16

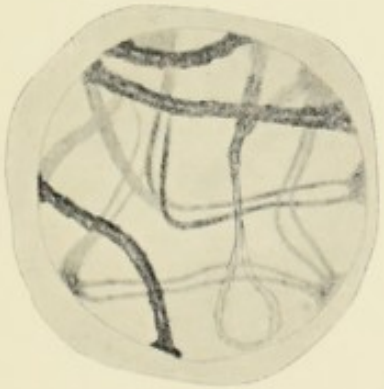

Fig. 17

Fig. 18

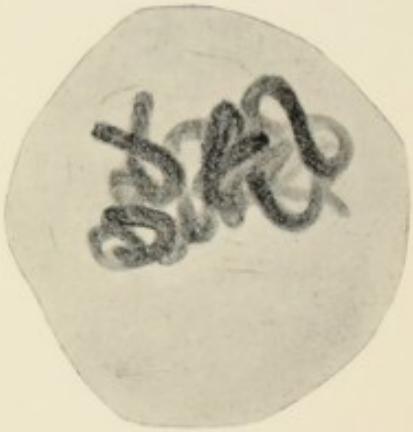

Fig. 19

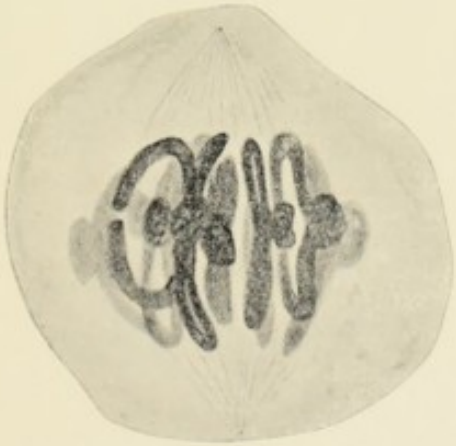

Fig. 20

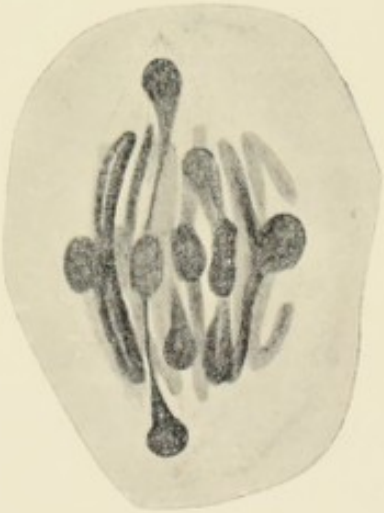

Fig. 21

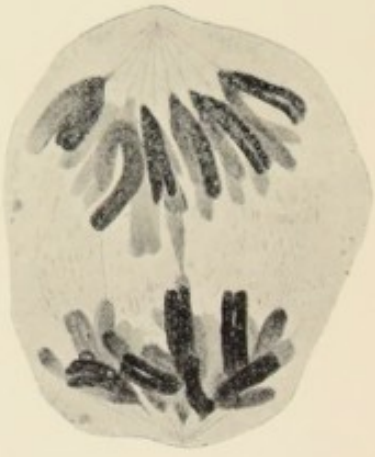

Fig. 22

7—ON THE EXISTENCE OF PERMANENT FORMS AMONG CHROMOSOMES OF THE FIRST MAIOTIC DIVISION IN CERTAIN ANIMALS.*

By J. E. S. Moore, A.R.C.S., F.L.S., Director of the Cancer Research Laboratories, University of Liverpool, and George Arnold.

[From the Proceedings of the Royal Society, B, vol. 77, 1906].

Many cytological investigators have drawn attention to the peculiar forms assumed by the heterotypical aggregates of chromosomes, or, as we have lately termed them, the *Synaptic Gemini*† of the first maiotic division. It was indeed the striking peculiarities presented by the forms assumed by these bodies, and the comparative ease with which they can be distinguished from the true chromosomes of pre- and post-maiotic mitosis, which led Professor Farmer‡ and one of us some years ago to make a list of the different forms we encountered in different animals and plants. We utilized this comparison in establishing what was at that time by no means clear, namely, that in regard to the maiotic divisions we are dealing with an identical process throughout both animals and plants.

In the note referred to and in subsequent publications by other authors, the view has been taken that the adult gemini (heterotype chromosomes) are to be regarded as capable of assuming any of the forms which two flexible rods can take. As for example, when bent round each other; lying parallel to each other; associated together in the form of a cross; joined end to end in the form of a ring; and so on. The rarer forms, such as tetrads, being accounted for by supposing that the four-fold figure results from a thickening or clubbing of the ends of the associated chromosomes thus:—= ⁙. In many instances where tetrads are found this is undoubtedly the explanation of their form.

Thus the possible modes of association between two flexible rods, together with the effects of alteration in their form, and the action of the spindle fibres, have hitherto been regarded by ourselves and others as sufficient to account for the great variety of aspect which the maiotic gemini are found to present.

In later years several authors have drawn attention to the obvious difference in size exhibited between one and another of the gemini on the same spindle. And not infrequently one or more of the gemini, under the title micro-chromosome or accessory chromosome, have been supposed to play an individual and important part in matters connected w th hereditary transmission and the determination of sex.§

* The title of the paper, as originally communicated and read, was "On the Constancy of Form among the Synaptic Gemini (Heterotype Chromosomes) in Certain Animals."

† Moore and Embleton, "On the Synapsis in Amphibia," 'Proc. R.S.,' 1906.

‡ Farmer and Moore, "On the Essential Similarities existing between the Heterotype Nuclear Divisions in Animals and Plants," 'Anat. Anzeiger,' 1895.

§ For example see Strasburger, "Typische und Allotypische Kerteilung," 'Jahrb. f. Wiss. Botanik,' vol. 42; O. Rosenberg, *op. cit.*, and others.

Our attention has latterly been redirected to the exact nature of the first maiotic (heterotype) division, owing to the fact that this form of mitosis has been found to occur during the development of malignant growths in man.*

In 1903, in conjunction with Professor Farmer, we were able, from an extended series of observations, to elaborate a general scheme of the maiotic process which appears to hold good throughout the higher animals and plants.† More recently we have dealt in detail over again and extended our observations upon the maiotic process in mammals and amphibia;‡ this revision having become apparently necessary, owing to the mutually divergent accounts of the maiotic process recently published by Strasburger and his pupils in relation to certain plants.§

A re-examination of mammals and amphibia has, however, confirmed our original standpoint with respect to the maiotic process in these vertebrates, and a similar confirmation has been given for plants in the case of Dictyotaceæ by Lloyd-Williams,‖ Gregory, and others. During the course of these revisions we have been led once more to the questions associated with the forms assumed by the gemini of the first maiotic division, and it appears to us now that the existing conceptions regarding this matter have become inadequate to meet the actual facts of the case. We do not at present profess in any way to have reached a final standpoint in our conceptions of the nature of the different forms of gemini, but the phenomena that have been brought to light are so remarkable in themselves, and from the point of view of theoretical developments so peculiarly attractive, that we desire to publish them in the hope that other cytologists may be able to throw fresh light upon the facts which we have already ascertained.

If the testis of *Triton* be examined at the time when the first maiotic division is abundant, groups of dividing nuclei may be found presenting the appearance given in fig. 1. The drawing is an accurate representation of a field of such dividing cells, and upon examination it may be found that the gemini (heterotype chromosomes) lie either upon young spindles or in groups within cells where the nuclear membrane has only lately disappeared.

If in such a field we consider some special form assumed by the gemini, such as the ∪, or ∝ ; it will be seen that it is generally possible to find this form in any individual cell we like to examine.

So also if we take another conspicuous class of gemini which may be represented thus:—Ð, we again find that this type also can be traced in almost any individual cell we please.

If for the time being we confine our attention to these two forms, and pass from the particular field represented in fig. 1 to a large number of similar fields, the conclusion is quickly forced upon us that the two forms in question are really always present in all first

* Farmer, Moore, and Walker, "On the Resemblances between the Cells of Malignant Growth and those of Normal Reproductive Tissues," 'Proc. R.S.,' 1903.

† Farmer and Moore, "New Researches concerning the Heterotype Divisions in Animals and Plants," 'Roy. Soc. Proc.,' 1903; Farmer and Moore, "On the Maiotic Phase (Reduction Divisions) in Animals and Plants," 'Quart. Journ. Micr. Sci.,' vol. 48.

‡ Moore and Embleton, "On the Synapsis in Amphibia," (*loc. sit.*); Moore and Walker, "On the Maiotic Phenomena in Mammalia," 'Thomson-Yates Reports,' University Press, Liverpool, 1906.

§ Strasburger (*loc cit.*). See also Overton, Miyake, and Allen, 'Jahrb. f. Wiss. Botanik,' vol. 42.

‖ J. Lloyd-Williams, "Studies in the Dictyotaceæ, I. and II.," 'Ann. Bot.,' 1904.

maiotic spindle figures. We soon see in fact, that the instances where one or the other is not conspicuous, are due to the particular gemini being turned in such a manner that they become foreshortened in the line of vision, or are obscured by other gemini, either totally or in part.

But besides the two forms of gemini above considered there are in *Triton* others. Thus we find, as cells in fig. 1 will show, gemini which present the appearance of two rods either lying parallel to one another, or crossed over one another, thus:—= ×. Again, there is a form consisting of a bent figure with symmetrical thickenings which form one aspect, may be represented thus:—(). Another form consists of an asymmetrical annulus with unequal sides, thus:—▷. While, lastly, we have a long and evenly thick ring, thus:—○. In this way it will be seen that in the first maiotic division of *Triton* there are to be found six varieties of gemini, and upon looking into the matter, we are led to conclude that all these six varieties co-exist in every instance of the first maiotic division.

In some cases, however, it is possible to see more than one representative of any particular type in one and the same cell, and upon counting the maximum representation of any type found in a particular element, we find in *Triton* this number is two. Taking each of the six classes or types of gemini in turn, we find that cells may be found that show two representatives of every one of the six classes.

In *Triton* the number of the pre-maiotic chromosomes is 24. These in the synapsis unite to form 12 gemini, and consequently we are driven to the conclusion that in the first maiotic spindle figure there exists a pair of gemini belonging to each of the six different types.

The fact that the varieties are constant in the early spindle figure, really in itself precludes the possibility of the different forms having anything to do with the fortuitous manner in which the gemini may become attached to the spindle fibres, and this indication is enforced to the point of proof by the further observation, which can be readily made, that all six varieties of gemini are present in cells before the nuclear membrane has disappeared, that is to say, before the spindle fibres have ever acted on them. In fig. 2 we have a drawing of five cells, in three of which the nuclear membrane is not yet ruptured, but in each of these three cells representatives of the different classes of gemini are as clearly to be discerned as they are on the early spindle figures.

Another feature which should be noted is the fact that we do not encounter transitional forms passing from one form of the gemini to another; there is no half-way set of gemini between the forms A and B given on the table on page , or between C and D, E and F, and so on.

The gemini in *Triton*, as we believe is the case in every other instance of the first maiotic division, are produced by the conjugation of pre-maiotic (somatic) chromosomes in pairs during the synaptic rest;* and since there are 24 pre-maiotic chromosomes in the particular instance of *Triton*, it follows that there must be only four individual chromosomes which can unite with each other to form the two gemini belonging to each of the six types.

* Farmer and Moore, 'Proc. R.S.,' 1903, *loc. cit.*; Farmer and Moore, 'Quart. Journ. Micro. Sci.,' vol. 48, *loc. cit.*; Moore and Embleton, 'Proc. R.S.,' 1905, *loc. cit.*; Moore and Walker, 'Thomson-Yates Reports,' *loc. cit.*

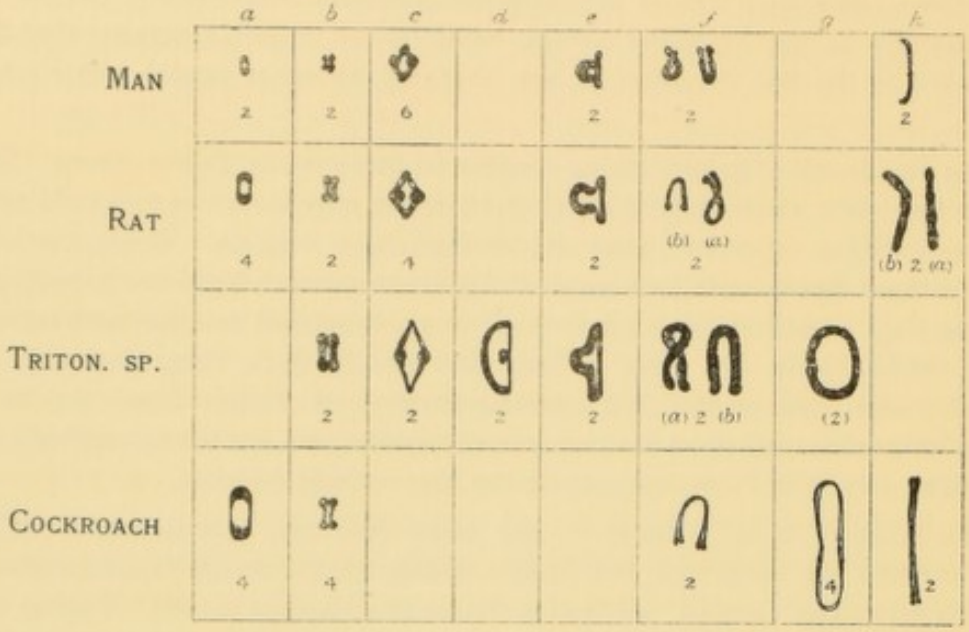

DIAGRAM SHOWING THE FORMS OF HETEROLYTIC GEMINI IN VARIOUS ANIMALS.

	Total number of gemini.		Total number of gemini.
Man	16	Triton, s.p.	12
Rat	16	Cockroach	16

The above results, based upon a study of the gemini in the first maiotic divisions in *Triton*, are interesting in themselves, but they immediately raise the further question as to whether the order here observed is simply a curious instance, or an individual expression of a wider law; on account of this we have studied in a similar manner the first maiotic division in man, rats, and *Periplaneta*; that is to say, in two more typical vertebrates and a representative arthropod.

In the testis of rats the first maiotic division occurs in groups of cells, and it is by no means difficult to bring under observation in a short time thirty or forty instances of the early spindle and late prophase. In this we have material amply sufficient to arrive at a decision upon the matters with which we are concerned. Fig. 3 is a drawing of a portion of a tubule from the testis of a piebald rat. It shows the outer wall of the tubule with some pre-maiotic nuclei and three dividing cells belonging to the first maiotic (heterotype) mitosis.

In these it will be seen that the gemini are of very different forms, and that as in *Triton* the same forms are repeated in different individual cells. Analysis of a large number of similar cells reveals the fact that in this particular example there are again six varieties of gemini (see table, page). In the rat, however, instead of the pre-maiotic chromosomes being 24 in number as in *Triton*, there are 32; consequently we shall have to ascertain the relative numbers of the different types. Further it will be seen that the six types present in the rat are not all the same as those catalogued for *Triton* (table, p.). It will indeed be obvious from this that only four of the Amphibian varieties are represented in the mammal; to these four two new forms of gemini are added.

In regard to the type H in the table, p. it is obvious that this particular form of the gemini might be regarded as an opened out **U**, which in this case appears as a straight rod; but although this is so, the fact remains that the types H and F are present together in rats in such a manner as to suggest that they are really distinct entities.

In rats we have said that the number of the pre-synaptic chromosomes is 32 and the number of gemini is 16. These latter bodies are grouped into six varieties, and consequently the number of each variety in rats must be unequal.

If in rats in a large number of division figures the maximum number of all the six varieties of gemini are counted, as was done in *Triton*, the results are as follows: A—4, B—2, C—4, E—2, F—2, H—2.*

Upon comparing the above results with similar results in man we find that here the varieties remain the same as in rats, but the relative numbers of these varieties are again changed (see fig. 6, and table, p.), the arrangement in man being as follows: A—2, B—2, C—6, E—2, F—2, H—2.

For any one species the numbers of types of gemini, so far as we have gone, appears to be constant, and the same types are retained in the case of fairly remote genera, such as *homo* and *mus*; but in these genera the relative numbers of the different kinds of gemini may vary with, or independently of, the number of the pre-maiotic chromosomes.

Passing from the above vertebrate example to the old arthropodean type *Periplaneta* we find, as fig. 5 and the table on p. will show, that here the number of the types of gemini is reduced from six to five.

Upon consideration of the table it will be seen also that three of the amphibian and mammalian types are retained, but no new type is added, and two of the types common to both the other groups are altogether wanting.

In *Periplaneta* there are 32 pre-maiotic chromosomes and 16 gemini, so that here, as in the case of man and rats, the number of similar forms must be unequal.

Counting the maximum number of any type in a number of cells, as was done in the former cases, we get the relative number of the five types in each cell as follows: A—4, B—4, F—2, G—4, H—2.

The possible bearing of the above observations upon the various existing theories of hereditary transmission, and especially in relation to the Mendelian hypothesis, will be obvious enough; but we feel a great reluctance at the present time in any way to augment the obscuration of the facts by putting forward crude theoretical anticipations.

What appears to us of first importance is the recognition of the actual existence of permanent structural types in the gemini of different forms. Secondly, it would appear that in any particular form the number of gemini of each type have a constant numerical relationship to each other. Thirdly, so far as the investigation has at present gone, certain types of gemini appear to be common to all the widely sundered forms examined. Still further, it will be seen that the number of different types of gemini is less in the oldest evolutionary form *Periplaneta*.

* It is an interesting and important fact that the number of pre-maiotic (somatic) chromosomes is not the same in rats as in mice. In the latter the number is 24.

Whether this last indication will be found to hold good is a matter upon which it would at present be useless to speculate; but the fact itself opens up a line of future inquiry which is certainly full of possibilities.

It seems to us, moreover, that it should be emphasized that both in regard to the permanent types of gemini and their numerical relationships, as well as with respect to the numerical constancy in the chromosomes themselves and their periodical reductions, we are face to face with constant arrangements in the parts of the unit of living substance (the cell) which seem to underlie and to be quite independent of those external interactions that are supposed to have helped to build the grosser features of living things.

With regard to the different types of gemini, it should further be pointed out, that the existence of these types implies substantive differences between the chromosomes that can unite to form the different kinds. It must be remembered that each of the gemini arises through an association of optically similar pre-maiotic chromosomes, but that at the time the nuclear membrane is about to disappear these associations have assumed different forms. They cannot do this unless they are of a different nature. The fact that there exist in those nuclei which we have examined groups of similar gemini shows that there must be sets of pre-maiotic chromosomes which in the synapsis can conjugate with each other, but not with the remaining individuals.

The present position may be in part summed up as follows:—In the fertilized egg the paternal and maternal chromosomes divide independently on the spindle of the first segmentation figure. And they go on dividing in a similarly independent manner throughout the soma, and during the pre-maiotic history of the reproductive elements themselves. In the synapsis which ushers in the maiotic phase the chromosomes unite in pairs, and in those cases we have as yet examined only certain individual chromosomes are capable of uniting with one another to form differing group of gemini; in each of these groups the number of gemini is more than one, and it varies in the different species hitherto observed.

Thus whether the conjugation of the chromosomes in the synapsis is really the final consummation, after many generations long delayed, of the copulatory intentions of the paternal and maternal elements, is a matter upon which there is as yet no actually conclusive evidence.

DESCRIPTION OF PLATE.

FIG. 1.—Groups of cells from the testis of a *Triton*. They are all in phases of the first maiotic (heterotype) division, and similar forms of gemini (heterotype chromosomes) are to be seen in numbers of different cells, as at *b*, *c*, *d*, *g*, *f*.

FIG. 2.—Cells from the testis of a triton, all in the late prophase of the first maiotic division. In three of the cells the nuclear membrane is still present; but the gemini have already assumed the same forms as those represented in fig. 1, *b*, *c*, *d*, *g*, *f*.

FIG. 3.—Cells from the testis of a rat, showing similar gemini, in the different cells, *a*, *c*, *e*, *f*, *h*.

FIG. 4.—Cell from testis of rat. Early phase from the first maiotic spindle, showing pairs of different gemini, *a*, *b*, *c*, *d*, *e*.

FIG. 5.—Group cells from the testis of *Periplaneta*, showing similar gemini in each of the three different cells, *a*, *b*, *f*, *g*, *h*.

FIG. 6.—Cells from the testis of man, showing similar gemini in the three cells at *a*, *c*, *e*, *h*.

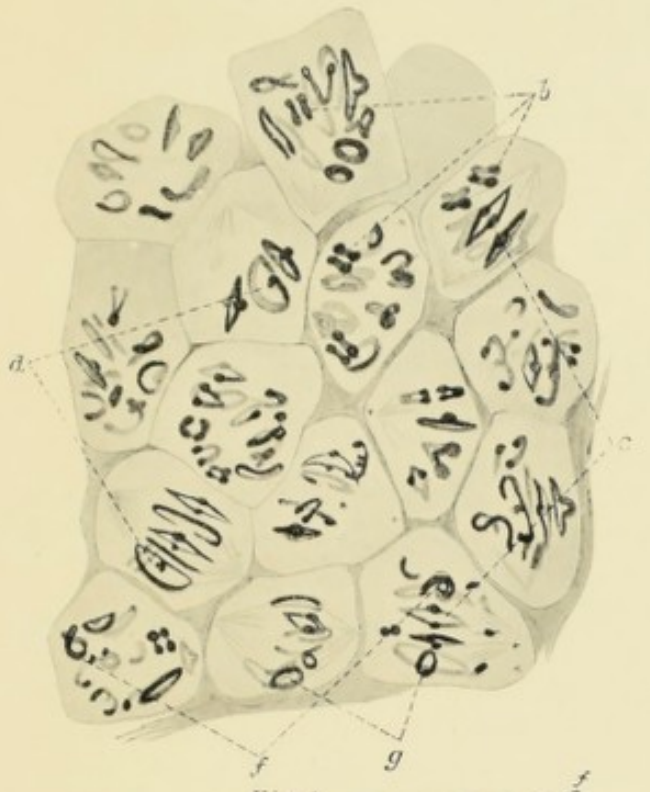

Fig. 1

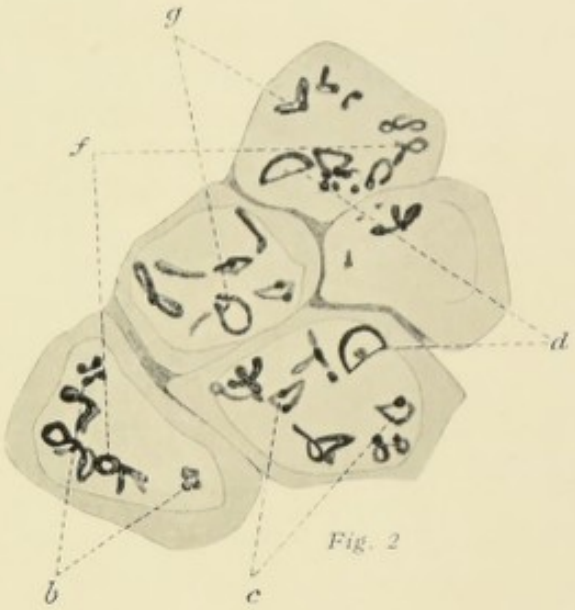

Fig. 2

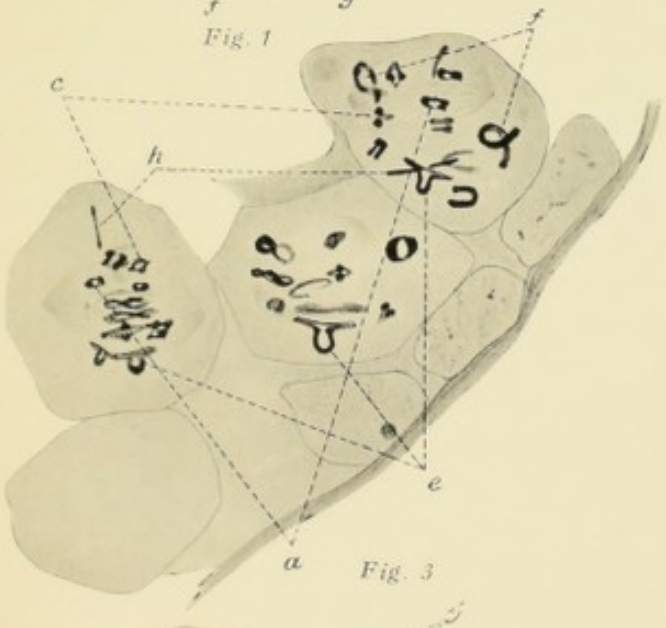

Fig. 3

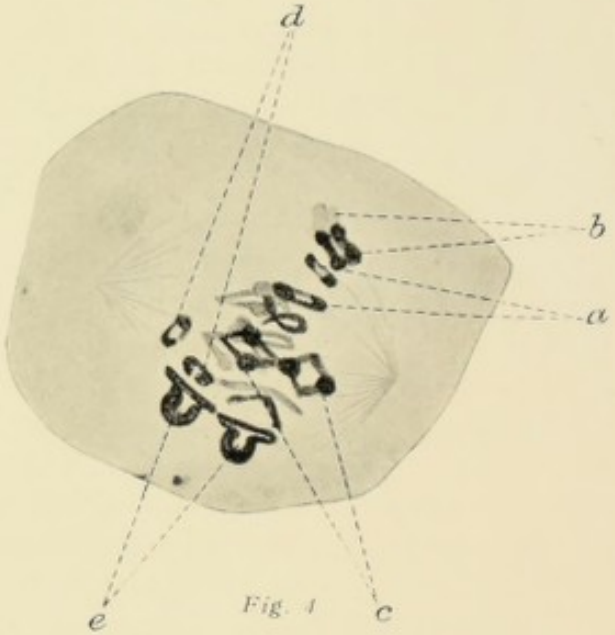

Fig. 4

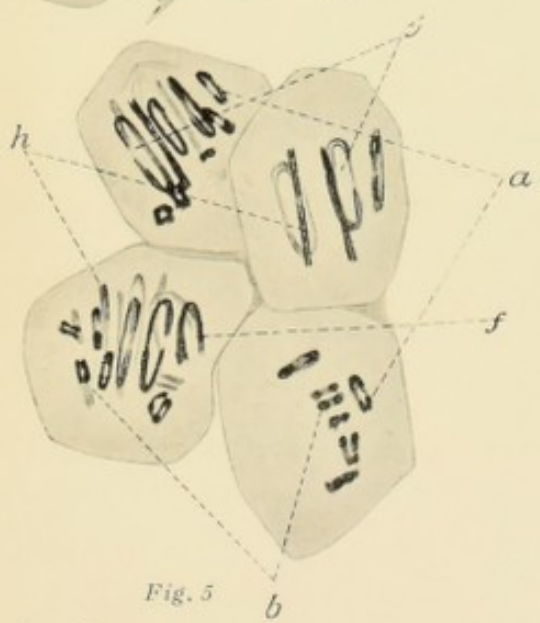

Fig. 5

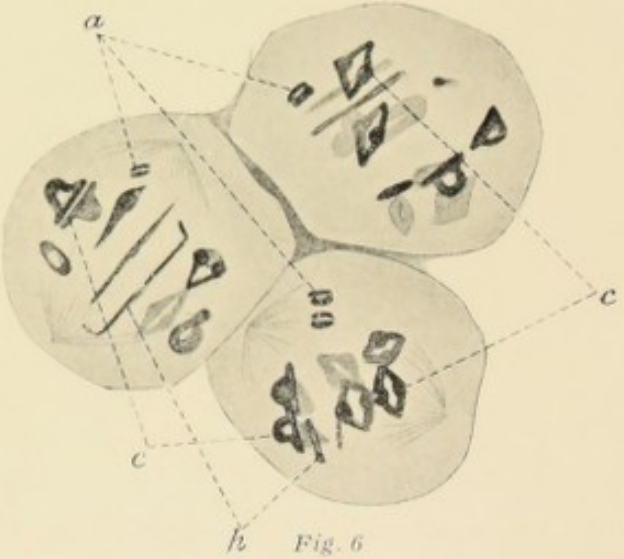

Fig. 6

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8—OBSERVATIONS ON THE LIFE HISTORY OF LEUCOCYTES.

By C. E. Walker, Assistant-Director of Cancer Research Laboratories, University of Liverpool.

(Received January 9—Read January 18, 1906.)

[From the Proceedings of the Royal Society, B, vol. 78, 1906].

In presenting this preliminary communication it is necessary to explain that the term leucocyte is used in the widest sense, and is intended to include all the wandering nucleated cells and their immediate ancestors. After many endeavours to make the observations here recorded compatible with the current classification of these cells, it has been found imperative to use a term that will include them all, and, at any rate in most cases, to put aside for the moment the question as to which particular kinds of leucocyte are being dealt with.

The tissues used have been chiefly bone-marrow, lymphatic glands and spleen, but the leucocytes in various other tissues and the free leucocytes in the blood have also been examined. Most of the work has been done with material derived from the guinea-pig and the rat, but tissues from man, rabbit, mouse, crocodile, frog, *Triton* and *Axolotl* have also been used. In every case the tissues have been normal, and with one exception derived from adult or nearly adult animals, the one exception being the testis of the early embryo of the guinea-pig.

In examining a section of bone-marrow one of the most striking, constant and frequent objects met with is the giant cell or myeloplax. In the myeloplaxes there is, as a rule, an appearance of amitosis in the nucleus or nuclei (see fig. 1).

Occasionally, however, a myeloplax is found in which mitosis is taking place, and in such cases the mitotic figure is pluripolar but otherwise of the somatic type (see fig. 2). A further search shows that myeloplaxes with the chromatin of the nuclei in the ordinary somatic spireme form are not uncommon (see fig. 3). On the other hand it is seen that the majority of the myeloplaxes divide amitotically. A portion or portions of the cell containing one or more nuclei are then separated off (see fig. 4).

Following the cells thus produced, one is forced to conclude that many of the smaller cells in the bone-marrow are derived from them. Among these are the cells commonly known as "polymorphic nuclear leucocytes" and many of the cells containing a single and more or less rounded nucleus (see fig. 5). The polymorphic nuclear cells apparently continue to divide amitotically for a number of generations. It is impossible in the present brief communication to illustrate all these stages, but fig. 5 shows two of the most striking.

At present it is impossible to discriminate between the cells arising amitotically and those arising mitotically from the myeloplaxes.

Among the mononuclear cells of the bone-marrow and the germinal areas of lymphatic glands, mitotic division figures are very frequent. They are also present, but less numerous, among cells found in the blood and the spleen.

Many of these mitotic figures are of the ordinary somatic type, and nuclei showing the usual somatic spireme are not uncommon (see figs. 6, 7 and 8).

More rarely a division figure such as those shown in figs. 12, 13, 14 and 15 is met with. In many of these it is extremely difficult to make out even a single individual chromosome.

In others, however, several can be made out quite distinctly. This is the case in figs. 12, 13 and 14. In fig. 15 the chromatin masses are much less clear, but even this is more distinct than is the case in a considerable proportion of such cells. Though it is not easy, on account of the small size of the cells and the difficulty of obtaining good fixation, to count the chromosomes in even a few among a great many cells, in those where anything approaching an estimation of the number is possible, it is evident that the number is much smaller than that found in the somatic division figures occurring in the same animal. That reduction in the number of chromosomes takes place in leucocytes has already been pointed out in a previous communication.*

It would seem, on comparing these figures with fig. 16, which is the first maiotic (heterotype) division from the testis of a guinea-pig, that these two forms of division are similar to each other, but vastly different from the normal somatic division figures found in the same animal (see fig. 7).

The probability that these division figures are similar to that occurring in the first maiotic division in reproductive cells does not, however, depend only upon the forms of the chromosomes,† their number, or their arrangement upon the spindle. Prophases such as those shown in figs. 9 and 10 are not infrequent. In these the so-called spireme is seen to be distinctly split and to differ in other ways from the ordinary somatic spireme (see fig. 6). In fig. 11 a corresponding stage in the prophase of the first maiotic division in the testis of a guinea-pig is given as a comparison.‡ Beyond this again, one occasionlly finds a cell in the diaster stage in which some, at any rate, of the chromosomes are longitudinally split (see fig. 17). This is typical of the first maiotic division.§ In so far as the leucocytes are concerned, I have hitherto found this type of division only in the bone-marrow.

The typically somatic division figures and those that I have interpreted as heterotypes, however, even when taken together, are less numerous than another form of division of which figs. 18 to 23 are illustrations. This form of division is extremely common in bone-marrow and in lymphatic glands. Hitherto I have not found it in any other adult tissue or in any other tissue that I have examined, with one exception referred to later.

It is obvious, as will be seen from the figures, which are taken from cells occurring in the bone-marrow and lymphatic glands of the guinea pig and rat, that the number of chromosomes is not more than half the normal somatic number, which is 32 in both cases. The shape of the chromosomes seems to vary slightly in different cells. Some are short, thick, and slightly curved rods (see figs. 18 and 22). Others are more or less oval, often very irregular. There seems to be every reason for regarding these divisions as similar to the second maiotic (homotype) division. Examples of this division from the testis of the guinea-pig are given in figs. 24, 25 and 26. The great frequency with which this division

* Farmer, Moore, and Walker, "On the Cytology of Malignant Growths," 'Proc. R.S.,' 1906.

† It may here be pointed out that several of the permanent forms of the first maiotic (heterotype) chromosomes described by Moore and Arnold have been observed in the division figures dealt with here. (Moore and Arnold, "On the Existence of Permanent Forms among the Chromosomes of the First Maiotic Division in Certain Animals," 'Proc. R.S.,' 1906.

‡ Farmer and Moore, "On the Maiotic Phase (Reduction Divison) in Animals and Plants," 'Quart. Journ. of Mic. Science,' vol. 48, Part IV., February, 1905; Moore and Walker, "The Maiotic Process in Mammalia," 'Thompson-Yates Reports,' University of Liverpool, 1906.

§ Farmer and Moore, Moore and Walker, *loc. cit.*

occurs, suggests strongly that there are several generations of cells showing the reduced number of chromosomes. The fact that in many cells the chromosomes are rod-shaped while in others they are roughly oval, led me at first to believe that there might be a well-defined difference between the first and the succeeding generations after the first maiotic division. An examination of the testes of several animals, however, convinced me that the same difference might be demonstrated among the homotypes there (see figs. 24 and 25). How far this is to be regarded as a real difference or merely the result of a presentation of the cell to the eye or to faulty fixation in some cells is here beside the point, as we know that in these particular animals there is no further division after the second maiotic (homotype).*

In comparing the foregoing observations with what occurs in the normal production of sexual elements in animals and plants, some remarkable similarities become evident. Amitosis occurs as one of the earliest known phenomena in the production of spermatozoa in some animals if not in all.† The first maiotic is, in spermatogenesis, immediately preceded by the ordinary somatic form of division. Then follows the second maiotic division, retaining half the somatic number of chromosomes as in the first maiotic. No further division has been recorded in the case of animals. Except as regards the latter point, what happens among the cells of the bone-marrow seems to be in many respects parallel to what happens in those of the testis, for the myeloplaxes very possibly, probably as I believe, correspond to the more or less syncytial condition of the cells sometimes observed in vertebrate testes, both arising amitotically.‡

In plants, however, we find that though the series of complicated cell phenomena that occur in the production of sexual elements are practically identical to what occurs in animals, other cells than those destined to become mature sexual cells are involved in the maiotic phase. Without going into details that would here be out of place, it may be pointed out that in many cases in plants but comparatively few of the cells that pass through the first maiotic division and thereafter show but half the somatic number of chromosomes, ever become converted into sexual elements, and also that the number of post-maiotic generations (those following the second maiotic or homotype division) is often very great, even if they can be considered in many cases as having any definite limit at all.

If the observations recorded above be correct, it would seem that the life-history of the leucocytes, in so far as I have been able to follow it, shows some remarkable points of resemblance to the life history of those reduced cells in plants to which I have just referred. This comparison is carried even further by what has been observed with regard to the origin and history of the foot-cells of the testis.* All the observations recorded above were made upon adult tissues, but in the course of seeking for the origin of the foot-cells, I examined the testes of very early embryos of the guinea-pig. Here, long before the formation of the tubules, the cells that are destined to become foot-cells are practically indistinguishable from certain of the stages observed in the leucocytes found in the bone-marrow and lymphatic

* Farmer and Moore, Moore and Walker, *loc. cit.*

† Meves, 'Anat. Anz.,' 1891, No. 22; *ibid.*, 94, 'Arch. m. Anat.'; Moore and Walker, *loc. cit.* Observations contained in a paper, not yet published, by Miss Embleton. They were carried out in the laboratories of the Cancer Research, University of Liverpool.

‡ Moore and Walker, *loc. cit.*

glands of the adult animal. In these cells, besides the ordinary somatic division figures, others are found exactly similar to those that I have described as second maiotic and post-maiotic in leucocytes (see figs. 27 and 28). In *Triton* testes it is possible to trace stages between the cells that have always been regarded as, and probably are, connective tissue cells, and the cells that apparently perform the same function as the foot-cells of mammalian testes. In *Triton* also, during the earlier stages of the maiotic phase, the cells that are destined to become foot-cells are similar to certain leucocytes in the same animal. Accepting these observations as correct, and regarding the pockets of the amphibian testis as being directly comparable with the tubules of the mammalian testis, I am forced to the conclusion that either the leucocytes themselves or their immediate ancestors may give rise to connective tissue, the former probably being what really happens.

While, as has already been said, there are remarkable points of similarity between the life histories of the leucocytes and those cells in plants which, though reduced, never become converted into sexual elements, it is also evident that there are some important points of difference. In plants, both the cells just referred to and those which are converted into definite sex cells have commenced the maiotic phase at the same time, and their immediate ancestry is common to both. In the case of leucocytes and certain connective tissues, it is not at present demonstrable that anything of the kind happens. It may be that our present conception of what constitutes the whole of the maiotic phase, in animals at any rate, is too limited, and that the development of the mesoblast is in some way involved in its earliest stages. In this connection the fact that in some plants reduced cells may be differentiated into tissues that are somatic in characters and function is extremely suggestive, as are also the observations of Loeb upon the segmentations and the production of embryos in unfertilised eggs.

It does not seem out of place to mention here the bearing that these observations have upon what happens in cancer. As has been shown elsewhere,† one of the earliest phenomena observed in the development of cancer is the fusion of a leucocyte with a tissue cell and the subsequent division of the cell resulting from the fusion into two daughter cells, each possessing chromatic elements derived partly from the leucocyte and partly from the tissue cell. That in the subsequent generations of the cells produced from this fusion the characters of both ancestors should appear, is exactly what would be expected. Among the cells of malignant growths all the forms of division here recorded as occurring among leucocytes and their immediate ancestors are to be found. As has also been stated before, some of the cells in malignant growths apparently go on dividing mitotically for a number of generations with the reduced number of chromosomes.‡

* Walker and Embleton, "On the Origin and Life-history of the Sertoli or Foot-cells of the Testis" (p. *supra*).

† Farmer, Moore and Walker, "On the Behaviour of Leucocytes in Malignant Growths," 'Trans. of the Path. Soc. London,' vol. 56, Part III., 1905.

‡ Farmer, Moore and Walker, "On the Resemblances exhibited between the Cells of Malignant Growths in Man and those of Normal Reproductive Tissue," 'Proc. R.S.,' December, 1903.

Note.—January 8, 1906.—The following fixatives have been used and in every case the results described have been found with all the fixatives. Flemming's Fluid (strong formula), Hermann's Fluid, Acetic Acid and Absolute Alcohol, Corrosive Sublimate and Acetic Acid, and strong Formic Acid. As it is possible that these observations may interest some who are not conversant with cytological methods, it is perhaps permissible to point out that the greatest care must be taken with the processes of fixation, dehydration, imbedding, staining, etc. Extremely small pieces of tissue should be placed in the fixative within about a minute of the death of the animal or removal from the living body. The dehydration should be carried out in short stages, an increase of 10 per cent. of alcohol at a time being perhaps best. This does not of course apply to the tissues fixed in Acetic and Alcohol or strong Formic Acid (40 per cent.), from which the tissues are transferred immediately to absolute alcohol. At the same same it is necessary that the tissues should not be left in under 80 per cent. of alcohol for more than two or three hours after fixation. In embedding, no higher temperature than 45° Centigrade should be used. These remarks apply particularly to mammalian tissues, but also to all animal tissues. Throughout the process of staining and mounting the greatest care must be taken that the sections do not become even partially dried upon the slides.

It is almost necessary to use a 10-inch tube microscope with a monochromatic light. I have used apochromatic objectives and eye-pieces specially constructed for the long tube by Zeiss. With a monochromatic light it is possible to obtain excellent definition with a 27 or even 40 compensated ocular and a 2 or 3 mm. apochromatic objective. Anything approaching this is impossible with the ordinary short tube.

In view of the enormous advantage gained by using a monochromatic light, the stains must be chosen with regard to the colour of the light used. The part of the spectrum between the blue and the green gives the shortest wave-lengths that can be conveniently used. As this gives a better definition than the parts of the spectrum with longer wave-lengths, red, yellow and orange stains give the best results.

DESCRIPTION OF PLATES.

FIG. 1.—Myeloplax from bone-marrow of guinea-pig, showing nuclei apparently dividing amitotically.

FIG. 2.—Ditto, showing pluripolar spindle figure.

FIG. 3.—Ditto, showing two nuclei with somatic spiremes.

FIG. 4.—Ditto, showing nuclei that have divided and the cytoplasm dividing (amitosis).

FIG. 5.—Adjacent cells in the bone-marrow of guinea-pig, showing stages of differentiation.

FIG. 6.—Mononuclear cell from bone-marrow of guinea-pig. The somatic spireme is formed.

FIGS. 7 and 8.—Later stages in the somatic type of division in similar cells.

FIGS. 9 and 10.—Prophases of division in cells of bone-marrow of guinea-pig, showing splitting of the so-called spireme thread.

FIG. 11.—Similar stage in the first maiotic prophase of division in the testis of guinea-pig.

FIGS. 12 and 13.—First maiotic (heterotype) division figures from the bone-marrow of guinea-pig.

FIG. 14.—Ditto from bone-marrow of rat.

FIG. 15.—Division figure from bone-marrow of guinea-pig.

FIG. 16.—First maiotic division figure from testis of guinea-pig.

FIG. 17.—Diaster stage from cell in bone-marrow of guinea-pig. Some of the chromosomes are longitudinally split.

FIGS. 18, 19 and 20.—Second maiotic (homotype) division figures from bone-marrow of guinea-pig.

FIG. 21.—Ditto from germinal area of lymphatic gland of guinea-pig.

FIG. 22.—View of equatorial plane of a division figure similar to those shown in figs. 18 to 21.

FIG. 23.—Diaster stage of a similar figure. Bone-marrow, guinea-pig. (Compare with fig. 8).

FIGS. 24, 25 and 26.—Second maiotic division figures from testis of guinea-pig.

FIG. 27.—Testis of embryo guinea-pig. A large male ovum and a homotype division figure among the cells which will form the foot-cells.

FIG. 28.—Ditto, showing somatic division figures.

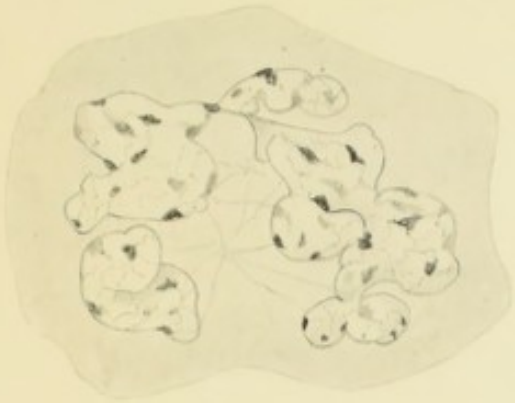

Fig. 1

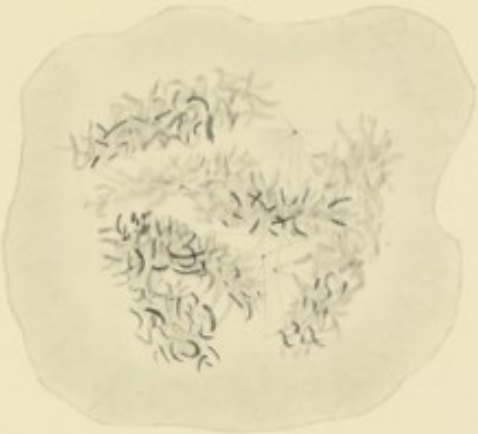

Fig. 2

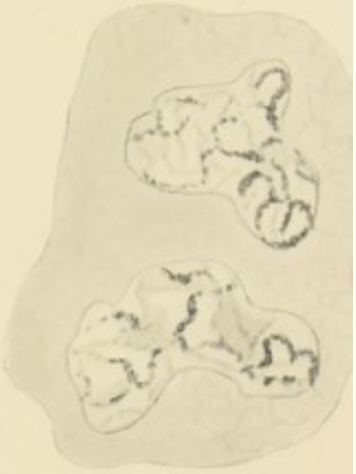

Fig. 3

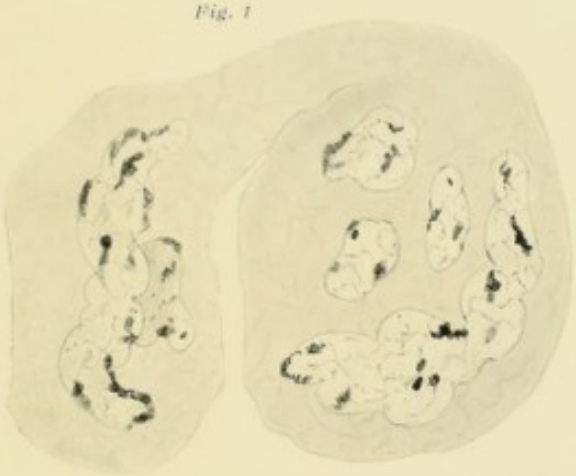

Fig. 4

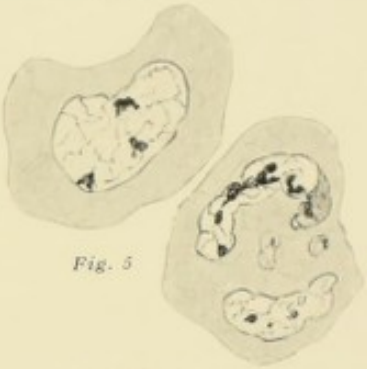

Fig. 5

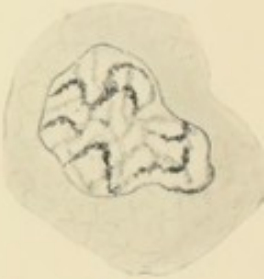

Fig. 6

Fig. 10

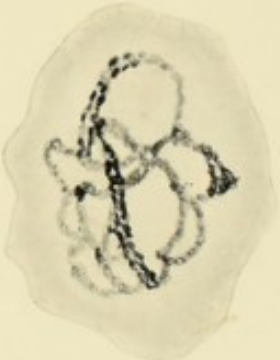

Fig. 9

Fig. 7

Fig. 8

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Fig. 19

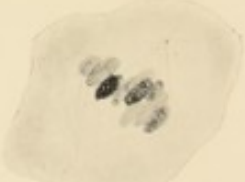

Fig. 20

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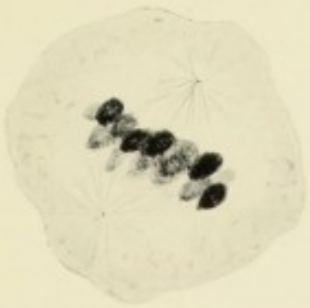

Fig. 21

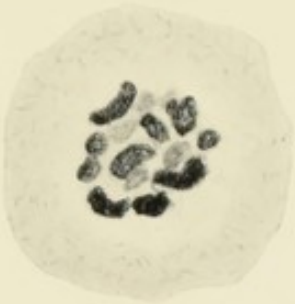

Fig. 22

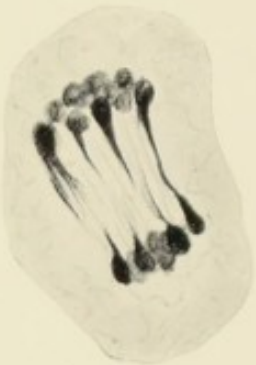

Fig. 23

Fig. 24

Fig. 25

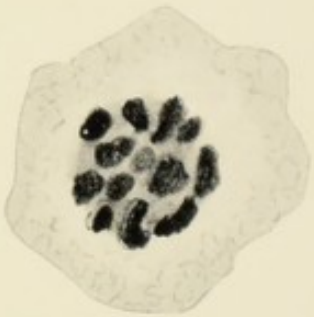

Fig. 26

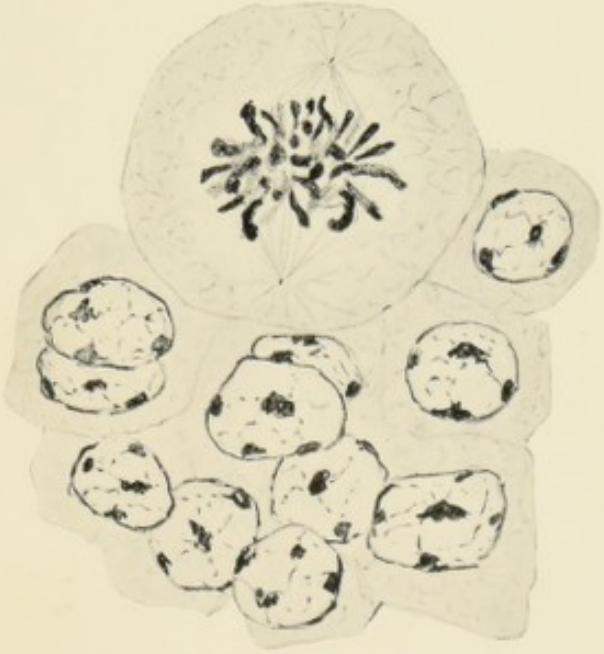

Fig. 27

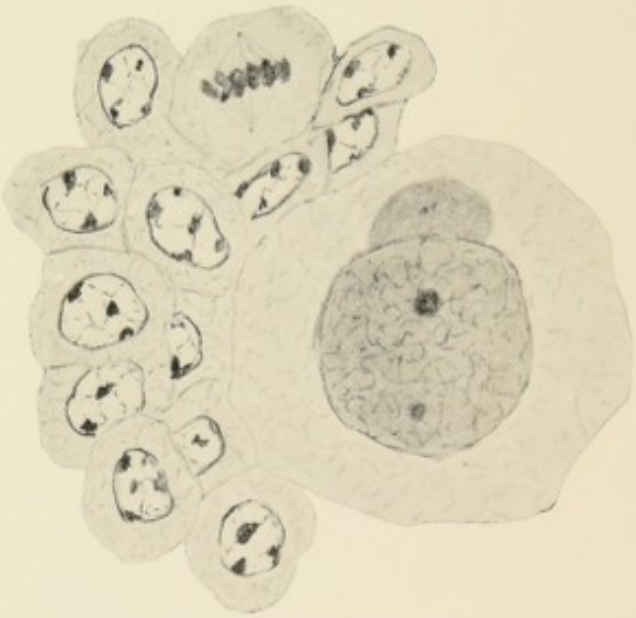

Fig. 28

9—ON THE ORIGIN OF THE SERTOLI OR FOOT-CELLS OF THE TESTIS

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and Miss Alice L. Embleton, B.Sc.

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The function of the Sertoli or foot-cells of the mammalian testis has frequently been described, and it is not intended to deal with it in the present brief communication. Neither do we intend to deal with the later stages of the life history of these cells, except in so far as to draw a parallel between them and the cells performing a similar function in the amphibia.

In the embryo testis of a mammal before the tubules are formed, it is seen that a number of so-called male ova lie singly or in groups of up to about four, among masses of cells, which we will for the moment designate as being of a more or less undifferentiated character. These undifferentiated cells are much smaller and of quite a different character to the male ova (see fig. 29).

At a little later stage we find that the wall of the tubule begins to appear. This wall is apparently actually in process of formation in parts of fig. 29, while in fig. 30 a tubule with a complete wall has been formed. We are convinced from a careful study of the stages of development that cells which form the wall of the tubule and those that are enclosed with the male ova inside the wall thus formed, are identical or derived from the same immediate ancestors. As development goes on these cells become more and more differentiated until we reach the state of the tubule in the adult testis (fig. 31).

The processes that are gone through in the testis of *Triton* seem to offer a further confirmation of the conclusions suggested above. Here the normal course of events in the male ova seems to be: (1) amitosis; (2) somatic mitoses; (3) the maiotic phase; and (4) the conversion of the cells resulting from the second maiotic (homotype) division into spermatozoa. These periods are sharply defined and are easy to follow. While amitosis is taking place, the individual male ova seem to be scattered amongst a number of "undifferentiated" cells, just as happens in the embryo testis of mammals. At this time the future pockets are but ill-defined or not defined at all. Apparently each individual male ovum eventually gives rise to a pocket (fig. 32). A little later on we see that the cells surrounding the male ova wander in between them as they multiply amitotically, until a stage is arrived at when it is impossible to say whether a particular one of them is going to become a cell forming the wall of a pocket or one of the cells which is enclosed within that wall together with the male ova (figs. 33, 34). To the latter the spermatozoa eventually become attached.

It will thus be seen that what happens in the development of the embryo testis of the mammal is parallel with what happens every year in the testis of *Triton*. Certain undifferentiated cells which surround the male ova are more or less differentiated along different lines, some apparently becoming cells to which the spermatozoa are attached during a certain period of time, others forming the walls of the tubules or pockets as the case may be.

It will be found that there are, among the undifferentiated cells in the early embryo testis of mammals, forms of division where the chromosomes are apparently reduced in number and different in shape to what is seen in ordinary somatic division. These closely resemble what has been described as a second maiotic (homotype) division figure (figs. 18 to 21) preceeding Appendix. It has been shown by one of us* that this form of division is very common in leucocytes in bone marrow, and lymphatic glands. While we have occasionally seen somatic division figures among these undifferentiated cells of the embryo testis, this form of reduced division is by far more common. In the male ova themselves at this stage the only mitotic figures we have seen have been typically somatic, and it is hardly possible to confuse the one kind of cell with the other.

The fact that we have what are apparently second maiotic divisions in cells which are obviously not destined to become sexual cells is in itself very suggestive, but when we come to compare the cells in which this happens with cells that are undoubtedly leucocytic in character, the suggestion is very much strengthened (figs. 35 and 36). We use the term leucocyte in the widest sense, and under it include all the wandering cells of the body.

Our conclusion is, therefore, that the foot-cells of the testis and the cells forming the walls of the tubules or pockets have immediately common ancestors, and that if these cells are not identical with certain stages in the series of leucocytic generations, they are derived from cells that were identical not more than two or three generations before.

* C. E. Walker, "Observations on the Life History of Leucocytes," 'Proc. R.S.,' 1906.

DESCRIPTION OF FIGURES.

FIG. 29.—Early stage in the development of the testis. Signs of the wall of the tubule are visible in places. A division figure among the undifferentiated cells is shown. (Embryo guinea-pig).

FIG. 30.—Later stage of the same. The wall of the tubule is formed.

FIG. 31.—Part of a tubule of adult testis, showing foot cells. (Guinea-pig).

FIG. 32.—Early stage in the testis of *Triton*. The walls of the pockets are not everywhere defined.

FIG. 33.—The same, showing the undifferentiated cells wandering in among the male ova.

FIG. 34.—The same, showing pockets is process of differentiation and one completely formed.

FIG 35.—Leucocytes in the lymphatic gland of guinea-pig.

FIG. 36.—Leucocytes in the spleen of *Triton*.

PLATE I.

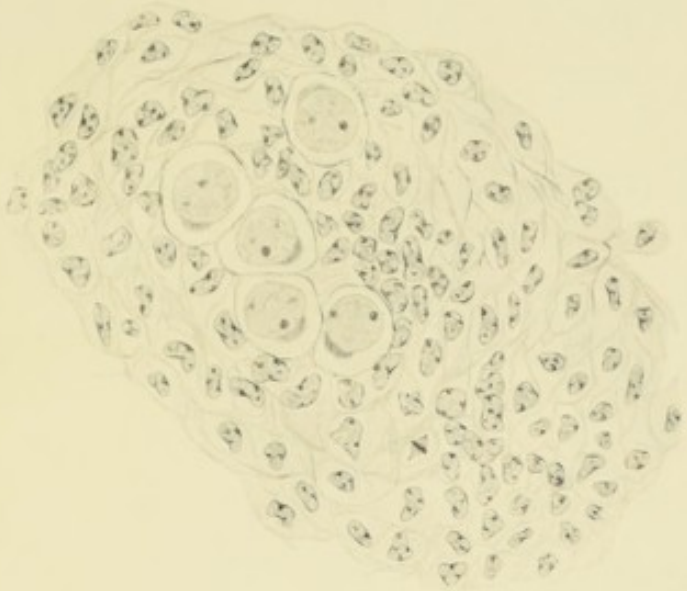

Fig. 29

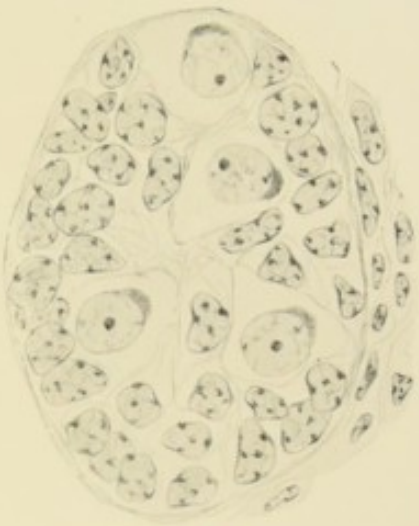

Fig. 30

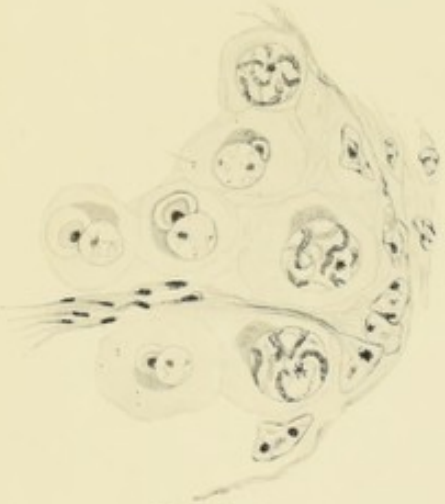

Fig. 31

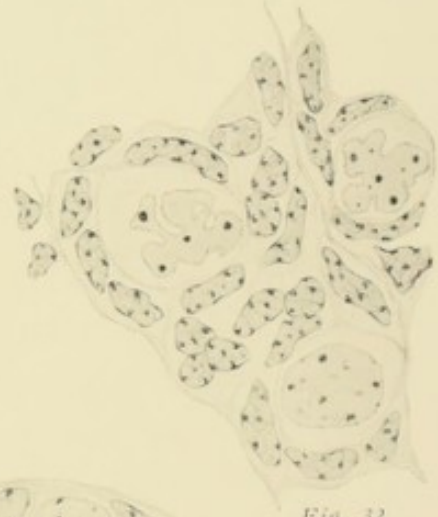

Fig. 32

Fig. 33

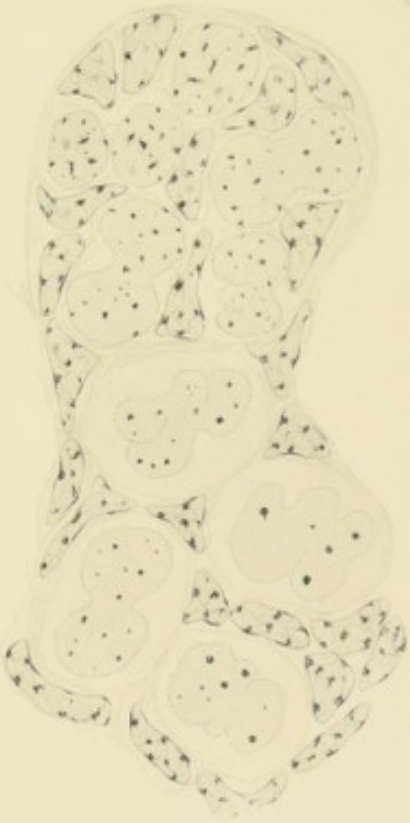

Fig. 34

Fig. 35

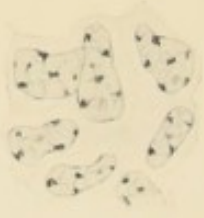

Fig. 36

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