different sub-cultures were experimented with at many different ages, and that in only two cases (viz., Experiments 8 and 97) was a pure Anthrax growth obtained when the infected water had been raised to 100° C., we conclude that any heating of Anthrax spores in water to this or any higher temperature, even for the shortest practicable time, is almost certain to insure their destruction.

What is the lower limit of destructive temperature, when the heating is prolonged, we have not attempted to determine, but we hope to make some observations on this point both with regard to Anthrax and some other spore-bearing bacilli, and to give the results in a further communication.

"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.

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 more remote ætiology of the disease itself.

We wish, however, at the outset, to disclaim all intention 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,

* 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.

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.

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 this 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 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.]

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 forms 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 cycle, 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 carcinomata 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 of 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, Shield, and others, who, by so kindly enabling us to obtain the necessary material, have made this investigation possible.