SOME POINTS OF DIFFERENCE BETWEEN HUMAN CARCINOMA AND TRANSPLANTED "MOUSE CANCER."¹

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(PLATE V.)

IN a recent communication (1911¹) a comparison was made between the number and shapes of the chromosomes found in the dividing cells of primary and secondary human carcinomata, human testis, and inflammatory tissue from man and the rabbit. The result of these investigations was to confirm the view, that not only is there a very similar and definite reduction in the number of chromosomes, both in primary and secondary human carcinomata and in the human testis, to half that of the ordinary somatic number, but also to show that some of the chromosomes in the reduced mitotic figures showed meiotic (heterotypical)² forms. On the other hand, in inflammatory tissue, both in man and in the rabbit, there is neither a definite reduction of the chromosomes to half the normal somatic number, nor can any meiotic forms be made out. There is a reduction, but it is irregular, pointing to irregular asymmetrical mitoses, such as were experimentally produced by Galeotti (1893³) and others.

In this paper a similar comparison between "mouse cancer," produced by grafting mouse testis and inflammatory tissue from man, will be made to show the difference in the cellular phenomena of primary and secondary carcinoma, as it occurs in man from those of transplanted "mouse cancer."

The object of this work is to see if transplanted "mouse cancer" shows the same cytological phenomena as do the human carcinomata (primary and secondary). This is an all-important point, for unless they both possess similar cell phenomena we are not justified in

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² The term "heterotype" is best avoided, as it has been used to describe any irregular as well as the normal reducing form of mitotic division. Throughout this paper, for the normal reduction of the chromosomes to half the somatic number, as is seen in gametogenic tissue, the term "meiotic" (Farmer and Moore, 1905²) will be used.

considering results obtained from experimenting with the one as bearing directly upon the other.

In 1903⁴, Farmer, Moore, and Walker first pointed out the regular symmetrical reduction of the chromosomes in cancer cells to half their normal somatic number, and gave a more detailed account of their observations in 1906⁵. Since the appearance of these papers many generalised contradictions have been advanced, but evidence of a kind sufficient to refute the statement has not been produced. Bashford and Murray (1904⁶) first confirmed the observations, and subsequently withdrew their confirmation (1906⁷); Klebs (1887⁸), Von Hanseemann (1893⁹), Galeotti (1893¹⁰), Deton (1911¹¹), and others have written on the subject, but none of them have published a systematic record of numbers and shapes of chromosomes, as recorded in the papers of 1911¹, 1906⁵. Of these papers those of Bashford and Murray necessitate further consideration. Their work was carried out almost entirely upon transplanted "mouse cancer," though the authors state (1904⁶, 1906⁷) that their observations hold good for primary carcinomata in the cat and trout also. They do not, however, give a description of mitotic phenomena as these occur in human cancer. They depict two cells from a squamous carcinoma of the tongue of a man, but make no reference to any counts of the chromosomes in human cancer. The object of their observations was presumably to inquire if meiotic mitoses were present in cancer, and whether such mitoses accounted for the reduced number of chromosomes observed in cancer cells. Their final conclusion was that meiotic mitoses did not account for the reduction in the number of the chromosomes, though they do not definitely refer to the question whether the reduction is usually regular or irregular. From their data they pronounce a verdict on the work of Farmer, Moore, and Walker, which, it is to be noted, had been done entirely on human cancer and without reference to cancer as it occurs in other animals, whether sporadic or transplanted.

The observations here described were undertaken for an entirely different purpose, for, as stated above, it is of prime importance that the similarity or dissimilarity between human and transplanted "mouse cancer" be established, otherwise we are working in the dark. With this aim in view, it was considered advisable to find out if meiotic mitoses occurred in transplanted "mouse cancer," as have been described as occurring in human carcinomata (1911¹, 1903⁴, 1906⁵).

For these investigations mouse testis and a transplanted "mouse cancer," given by Professor Ehrlich to the Cancer Research Laboratory in the University of Liverpool, were used. To eliminate any phenomena which might be due to inflammation, the tumour tissue chosen was taken from grafts before there were any signs of superficial ulceration, or degeneration in the centre of the tumour. Furthermore, from such growths the proliferating tissue at their periphery was taken, as here the vital processes of the growth are best marked, and there is less chance of including degenerating cells, which are usually first seen in the centre.

The points to which chief attention was paid were the number and shapes of the chromosomes, whether or not multipolar figures were present, and whether the dividing cells showed any definite inequality of the grouping of the chromosomes at their poles.

The method employed in recording the numbers and shapes of the chromosomes is the same here as in the previous papers (1911¹, 1906⁵). As it is only by counting the chromosomes in every cell possible in a number of sections, and taking an average of the cells counted, that we can come to any decision whether there is a reduction in the number of the chromosomes, and whether it is regular or irregular, the chromosomes in 200 and 100 dividing cells of the transplanted "mouse cancer" and the mouse testis respectively were enumerated. The taking of an average over a large number of cells helps to eliminate errors in counting, due to such causes as some of the chromosomes of one and the same figure being in consecutive sections, one chromosome being superimposed on another, and some chromosomes splitting before others and thus giving too high a count. These difficulties are the more obvious when the minuteness of the chromosomes in the mouse is considered. Profile views of mitotic spindles have frequently been neglected, as here one cannot always determine with any degree of exactitude the number of chromosomes present, but these views have been made use of in searching for meiotic shapes. Neither were counts made in the cells where the chromosomes were becoming clumped together prior to the formation of the daughter nuclei, so that the crowding together, pointed out by Bashford and Murray (1906⁷) as perhaps accounting for some of the recorded reductions in the number of chromosomes, does not operate in the counts recorded in this paper or in the papers previously referred to (1911¹, 1903⁴, 1906⁵). Further, to avoid any biased counting, as, for instance, counting a doubtful 25 in a cell that should probably only contain 24 chromosomes, and so giving the benefit of the doubt to the 24, the chromosomes in all the cells used in these observations were carefully drawn without being counted, the numbers and shapes of the chromosomes being thus registered for good, and not counted up or examined further until all the drawings needed were completed. Thus, until the graphs were made, one could have no definite idea of the ultimate result. Very special search was made for meiotic forms. Some of the chromosomes which divide in the ordinary somatic fashion, after splitting longitudinally, may remain attached by the open ends of the V's, the apices separating widely, and so simulate somewhat one of the meiotic shapes (see Plate V. Figs. 1-6), but any one familiar with such resemblances can readily distinguish them from the genuine meiotic chromosomes, not only by their size, amount of contained chromatin, and general appearance, but also by the absence of other meiotic forms in the same cell or other cells of the specimen, and by noting the whole number of chromosomes in the cell. In fact, the general appearance of a meiotic mitosis is so different from a somatic mitosis that it is not easy to mistake one for the other.

The so-called meiotic shapes in transplanted "mouse cancer"

figured by Bashford and Murray (1904⁶) fail to show true meiotic forms, and it is difficult to accept the later corrections of their views (1906⁷), based on the restaining of the specimens used to show meiotic forms in their previous paper. An examination of their figures depicting the same cells, as originally stained and after restaining, shows the outline of the cells to be much altered, the position in the cells of the chromosomes as a whole and the angles of the spindle fibres to one another being changed. This applies to all their restained figures in transplanted "mouse cancer." It is difficult to see how staining can produce such differences without materially altering the finer cell structures, in which case observations on such cells are, of course, worthless. Other factors they put

forward as causing "apparent" meiotic mitoses are variations in the shapes, position, and mode of attachment of the chromosomes to the spindle fibres, and the effects of apparent changes in the shape, brought about by the angle of presentation to the optical axis. Even if other observers have not made allowance for such factors, the appearances of chromosomes under these conditions show no true likeness to meiotic forms.

from the testis of a mouse. Here we see two definite maxima, one at 12 and the other at 24, the number of cells with 12, half the normal somatic number of chromosomes in the mouse, being more than twice those with 24, as we should expect from the process of spermatogenesis explained in a previous paper (1911¹).

Quite different is the graph formed by counting the number of chromosomes in 200 dividing cells, from Ehrlich's transplanted "mouse cancer" (Chart II.). Here we have a fair number of cells with 24 chromosomes, very few above this number, while a large number of cells show a reduction in the number of chromosomes, a reduction not regular by any means, as a glance at the graph shows. We have no distinct maximum at 12, as is the case with mouse testis. The fewness of cells with more than 24 chromosomes is emphasised by the fact that none of the cells seen in profile view

In Chart I. is represented the numbers of chromosomes in 100 cells

showed any very marked grouping of more chromosomes at one pole than the other, nor were multipolar figures observed.

There has been some difference of opinion regarding the normal somatic number of chromosomes in the mouse. Sobotta (1895¹²) first put the number at 24, others put it higher; thus Michaelis (quoted by Murray) thought it was thirty-six, and Murray thirty-two (1908¹³). In 1907, Sobotta (¹⁴) corrected his previous view and seconded Murray's observations. From a consideration of Chart I., there seems little doubt that the normal somatic number is 24, and this is further borne out by the maximum at 12, half that number, as we should expect from a regular reduction. Few cells with more than 24 and none with more than 28 chromosomes were observed. This carries great weight when we consider that it applies to 100 cells. From the numbers present in "mouse cancer" it would not be fair to pronounce any opinion, except that the number is probably not greater than 24.

The counts of the chromosomes in 100 cells of lupus vulgaris of the face, published recently, are reproduced in Chart III. We have one maximum at 32, the normal somatic number. There is also a reduction, but it is very irregular, with no definite maximum at 16. No likeness is seen between this

CHART II.—Transplanted "Mouse Cancer."

graph and that of mouse testis (Chart I.), but there is a similarity between it and transplanted "mouse cancer" (Chart II.). This similarity is more apparent when we consider that in Chart III. the irregular reduction is scattered over a larger range of figures (9 to 37) than in Chart II. (10 to 27), and that the number of cells in Chart III. is half that of Chart II.

Charts IV. and V. are reproductions of the graphs representing the number of chromosomes in each of 100 cells, from a rapidly growing primary carcinoma of the cervix uteri and a secondary epithelioma of the groin respectively. In the case of the primary carcinoma there was little or no inflammation superadded, and the graph shows a striking resemblance to normal gametogenic tissue (Chart I.), two well-marked maxima being present at 16 and 32, pointing to a symmetrical reduction in the chromosomes to half the

normal somatic number. The secondary carcinoma (Chart V.) shows similar maxima, but in this case there was a great deal of ulceration, so, as is to be expected, inflammatory phenomena mask this graph

CHART III.—Lupus Vulgaris of Face.

somewhat; however, it still retains the features of reduction already described as occurring in human carcinoma (1911¹, 1903⁴, 1906⁵).

A consideration of the above graphs (Charts I.-V.) shows a similarity between transplanted "mouse cancer" and inflammatory tissue, where in both cases the reduction in the number of chromo-

CHART IV.—Carcinoma, Cervix Uteri (rapidly growing).

somes is irregular. On the other hand, in human cancer, both primary and secondary, the latter being included because transplanted "mouse cancer" is looked upon as being an artificial metastasis, there is a symmetrical reduction to half the normal somatic number, as is

seen in mouse testis, and in fact in human testis and gametogenic tissue generally, though in this particular case of secondary human cancer the inflammatory process to some extent masks the meiotic phenomena.

The difference between transplanted "mouse cancer" and human carcinoma becomes still more marked when we consider the meiotic forms of chromosomes which have been worked out in gametogenic tissues. Some of the meiotic forms found in the testis of the mouse are shown in Plate V. Figs. 1 and 2. In each of 23 out of the 100 cells counted in the mouse testis more than two of these shapes were easily discernible. In some of the cells all or nearly all the chromosomes showed meiotic forms very clearly. In transplanted "mouse cancer" none of the cells examined contained any meiotic shapes. A few cells—10 out of 200—had 1, and occasionally 2, chromosomes, resembling, in the manner already mentioned, one of the meiotic shapes (Plate V., cf. Figs. 1, 4, and 6*b* to Figs. 3 and 6*a*), but no true meiotic shapes could be found. This one shape was more slender than a real meiotic form, and the number of chromosomes present

CHART V.—Epithelioma of Groin (secondary ulcerated).

in these cells also militated against their being regarded as meiotic mitoses. This apparent shape is probably due to a V-shaped somatic chromosome splitting longitudinally, and the two halves so formed not having completely separated, but still remaining attached by the open ends of the V's (Plate V. Fig. 6*a*). Bashford and Murray (1906⁷) have referred to these apparent forms, and illustrate some due to faulty staining, but a careful examination of many sections variously stained has failed to reveal any apparent figures beyond the one mentioned. In inflammatory tissue the chromosomes were very similar to those seen in transplanted "mouse cancer," no meiotic forms being observed in any of the specimens examined, though clumping together of the chromosomes and irregularities were comparatively common (1911¹). Human carcinoma presents an entirely different picture; meiotic shapes have been recorded by Farmer, Moore, and Walker (1903⁴, 1906⁵), and lately confirmed (1911¹) (see Plate V. Fig. 4).

From the foregoing observations, it may be seen that in transplanted "mouse cancer" the cells dividing by mitosis do so in a somatic manner. The reduction present is irregular, multipolar

figures are absent, the asymmetry of division is not marked, and is easily explicable by the casting out or degeneration of chromosomes and other phenomena known to occur as the result of pathological conditions generally. No meiotic shapes are seen. In all these details, transplanted "mouse cancer" bears a very close relationship to inflammatory tissue, not only in man, but also in the rabbit (1911¹). On the other hand, to mouse testis, transplanted "mouse cancer" does not present any similarity, for mouse testis shows a symmetrical reduction of the chromosomes to half the normal somatic number, and the presence of definite meiotic shapes, just as occurs in human testis, human carcinoma, and gametogenic tissue generally.

Not only do transplanted "mouse cancer" and human carcinomata (primary and secondary) differ as regards the phenomena seen in their individual cells, but when we consider the growths as a whole we see other differences. In the first place, the transplanted "mouse cancers," produced by grafting with Ehrlich's "mouse cancer," are limited by a definite capsule, and do not show that indefinite limit so familiar in human cancers. They attain an enormous size in relation to the size of the animal, and yet infiltration of the surrounding structures is not seen, nor apparently is the general health of the animal affected. Pressure from the increasing size of the tumour may cause the skin to ulcerate over it, or the peritoneum to give way at some spot, but even here the peritoneum does not become studded over with growths, or the abdominal organs infiltrated, but adhesions are formed so that the capsule is practically completed again. Several observers (Bashford, Murray, and Cramer (1905¹⁵), Murray (1908¹⁶), and Haaland (1908¹⁷)) have described infiltration of the tissues surrounding other transplanted "mouse cancers," but these cases may be due to the separation of some of the "cancer cells" at the time of injecting as described below, or to the advance of the growth by pressure atrophy of the surrounding tissues. Certainly the illustrations of infiltration by transplanted "mouse cancers" are not very convincing, and the number of cases reported is very small indeed when we consider the enormous number of transplanted "mouse cancers" examined. Only comparatively rarely have "metastases" been found, and apparently only when emulsions of tumour cells have been injected. When the "mouse cancer" is grafted the cells of the graft tend to remain together during and after their insertion, so that there is comparatively little chance of some cells being carried off by a small blood-vessel injured in the operation of insertion. It is quite a different matter with the injection of an emulsion of "mouse cancer" cells hypodermically or intraperitoneally; the cells here are more or less separated, they are very minute, and the chances of some of them finding their way into a small blood-vessel opened by the needle are considerable. Not only is this so, but being forced by the syringe into the subcutaneous tissue or peritoneal cavity, they will

readily pass in any direction wherever they can find an opening. The ease with which these "cancer cells" in emulsion can spread is well illustrated by a case published lately by Haaland (1911¹⁸), where, after an intraperitoneal injection, several foci of growth were found dotted over the peritoneum, but this does not prove a metastatic element to have been present. Further, the injection of emulsified cells, employed by many for the propagation of "mouse cancer," may quite well explain how some cells, separated at the beginning from the main mass of cells, may develop separately a little way off and thus be mistaken in sections for infiltration by the main growth, and also how emboli of these cells will probably lodge in the lungs by gaining access to the blood-vessels at the time of their inoculation into the new host.

As regards "metastases," they have always been in the lungs, having probably travelled there by the pulmonary artery, and on the few occasions on which they have been present they have been small. How different is the spread in the human subject, where the lymphatic glands and the liver are frequently the sites of secondary growths, while, when the lungs are affected, metastases in other parts of the body are usually present also, and in many cases the growths are in the pleura in the first place, and spread from here into the lung, having reached the pleura by the lymphatics. Moreover, the formation of secondary growths is common in human cancer. From this we see that whereas carcinoma in man spreads mainly by the lymphatics, in transplanted "mouse cancer," if it really occurs, the spread is by the blood-vessels, and from its occurrence only when an emulsion of "mouse cancer" cells has been used, suggests strongly that these so-called "metastases" of transplanted "mouse cancer" are not derived from the main subcutaneous or peritoneal tumour, but from the "cancer cells" inoculated at the same time.

In other transplanted "mouse cancers" different observers have reported "metastases" in the lungs. Borrel (1903¹⁹) was the first to record microscopic growths in the pulmonary artery in the lungs of a mouse inoculated with an adeno-carcinoma. Haaland (1905²⁰), Murray (1908¹⁶), and others have likewise described and figured "metastases" in the lungs, and in all cases apparently spreading by the pulmonary artery. One point we should note is that all observers seem agreed that the so-called "metastases" of transplanted "mouse cancer" travel by the blood-vessels in contradistinction to what happens in human carcinoma in which metastases usually take place through the medium of the lymphatics.

In conclusion, it must be quite clear that this paper does not touch on sporadic cancer in mice in any way. It is limited to transplanted "mouse cancers," such as are used in many laboratories. The Ehrlich tumour used in these observations is of rapid growth, is readily transmissible, and has been generally accepted as "mouse

cancer." With regard to its behaviour clinically, we cannot with justice compare it to human carcinoma, while cytologically it bears a far closer relationship to inflammatory tissue than to human carcinoma. At present one cannot say what the exact significance of this may be, but can only suggest that other so-called "mouse cancers" be carefully examined to show not only gross, but minute points of similarity to human carcinoma before deductions from the results obtained by experimenting with the one are accepted as being applicable to the other.

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DESCRIPTION OF PLATE V.

- FIG. 1.—Cell from testis of mouse, showing meiotic form of mitosis.
- FIG. 2.—Cell from testis of mouse, in late prophase of first meiotic division. Chromosome are beginning to assume definite shapes.
- FIG. 3.—Cell from transplanted "mouse cancer." Shows somatic type of division, one chromosome somewhat resembles a meiotic form.
- FIG. 4.—Cell from a carcinoma of the uterine cervix (human). Shows meiotic type of division. Note difference to Fig. 3.
- FIG. 5.—Cell from a carcinoma of the uterine cervix (human). Somatic type of mitosis, showing a similarity to Fig. 3.
- FIG. 6.—Diagrams representing (a) somatic type of chromosome which has split longitudinally, but the two V's have not completely separated. Note difference to (b) meiotic type. Chromosomes shown in full view and profile.