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HETEROLOGOUS TRANSPLANTATION. MOUSE TUMOURS IN RATS.

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Reprinted from THE LANCET, *April 3, 1915*

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MOUSE TUMOURS IN RATS.

WHEN a tumour arising in one species of animal is inoculated into an animal of another species the tumour fails to grow progressively. This specificity has in some cases been found to be so marked that a tumour occurring in one variety of mice will not grow in another variety. Throughout the literature of the experimental investigation of cancer there may be found repeated references to these facts, which must be taken into consideration in any theory of cancer. J. B. Murphy[1] has shown, however, that rat and mouse tumours may be grown by inoculating developing chick embryos. The inoculation is made on the fifth to the eighth day of incubation. The transplanted tumour fragment grows vigorously up to about the seventeenth day and then begins to be absorbed. Thus we have here a remarkable and conspicuous exception to the rule of specificity already noted.

It has been shown by Rywosch[2] that chick embryos have practically no bactericidal or bacteriolytic powers; and, *a priori*, one would suppose that the explanation of the success of the transplantation of rat and mouse tumours to chick embryos is to be found along similar lines—namely, the absence in the developing chick of the mechanism by which resistance to tumours of a foreign species is produced. The phenomenon has also been explained by assuming that the embryo contains an excess of "growth substances" which are not yet differentiated and which can thus provide sufficient of these necessary materials for the proliferation of the tumour cells.[3]

It seemed reasonable to anticipate that the mammalian embryo will provide as suitable a host

for a tumour of another mammalian species as the more distantly related chick embryo, and it is with this possibility that the present paper deals. A brief outline of the work has already been given in July, 1914, in the Twelfth Annual Report of the Imperial Cancer Research Fund.

The technique by which developing chicks are inoculated is very simple, but the difficulties of inoculating mammalian embryos through the uterine wall without bringing gestation to an end are very great, though it has been done.[4] It was therefore decided first of all to inoculate new-born rats with mouse tumours. It is to be noted that when a freshly hatched chick is inoculated with a rat tumour the tumour fails to grow, and it might be thought, by analogy, that a mouse tumour would not grow in new-born rats. But this was found not to be so.

The rat, under 24 hours old, is inoculated by the needle method, preferably in the subcutaneous tissue of the back. Occasionally the needle strikes a vein and a fair amount of blood is lost, but only one animal in several hundred was killed by the actual inoculation. The transplantation must be carried out quickly and the animals must be kept warm. The chief difficulty in this work lies in rearing the young rats. The mother is very liable to kill her young after they have been interfered with, but these accidents may be very much reduced by handling the mother rat frequently during the period of gestation.

The tumours inoculated were a polymorphous sarcoma (37), a carcinoma (63) which in mice grows progressively until it kills the host, and a carcinoma (206) which grows for about 16 days and then disappears, leaving the mice in an immune condition. With each of these tumours it was found that rapid growth takes place in rats inoculated during the first day of life for from 10 to 17 days, the tumour then disappearing, as illustrated in the charts reproduced. (Charts A, B, C.) From a dose of 0·01 c.c. it is easy to obtain in 10 days a tumour weighing over 1 gramme. (Fig. 1.) The mass consists of healthy tumour cells, showing in microscopical sections their characteristic shape and arrangement, with numerous mitotic figures. After the tenth to seven-

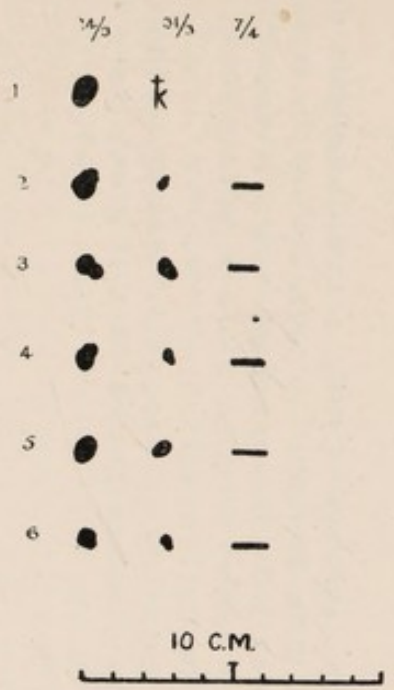
CHART A.

New-born rats inoculated on 30/3/14 with mouse carcinoma (63); dose 1·01 c.c. Scale as in Chart C.

CHART B.

New-born rats inoculated on 29/4/14 with mouse carcinoma (206); dose 0·01. Scale as in Chart C.

CHART C.

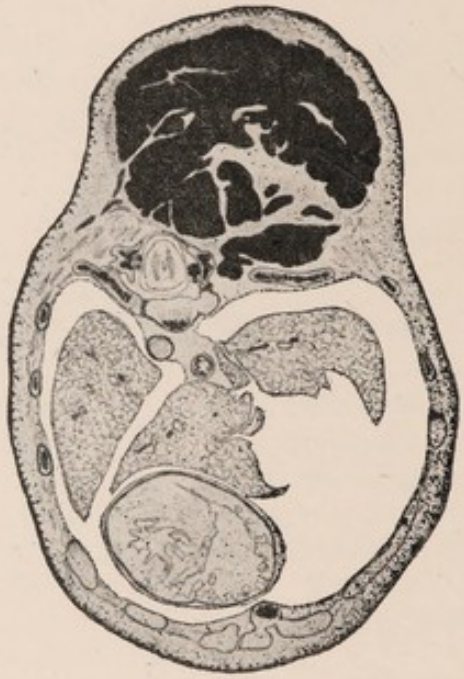


Half-grown rats, average weight 60 grammes, inoculated 17/3/14 with mouse sarcoma (37); dose 0·02 c.c.

teenth day increasing numbers of fibroblasts grow in thick strands from the capsule into the tumour, the tumour cells becoming necrotic. Eventually in a further period varying from one to two weeks the whole tumour is absorbed.

With regard to the sarcoma it was found to differ from the carcinomata in that it grows very well

FIG. 1.



Section through the thorax of a young rat 17 days old, bearing a mouse sarcoma. The tumour (black) is penetrating between the spine and the muscles. Small areas of necrosis and bands of fibroblasts are found in the tumour; in the healthy parts of tumour mitoses are numerous.

also in half-grown and adult rats. Russell[5] has shown that the cells of a mouse carcinoma survive and multiply in the normal adult rat for nine days; but the extent of this growth is insignificant compared with what has been obtained from minute grafts of the polymorphous sarcoma (37). Chart C indicates the size of the tumours obtained with doses of 0·02 c.c. of this sarcoma.

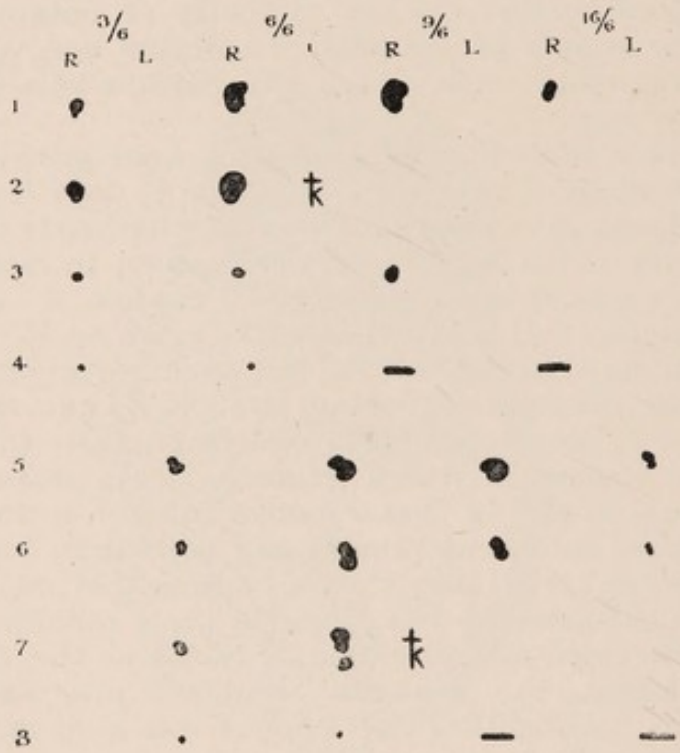
The attempt has also been made to cultivate this sarcoma in series both in new-born and in half-grown rats. In the former case the attempt was partly successful; the tumour was maintained 49 days for five generations in rats, and was then lost through the mother eating the young animals. The growths of the third and fourth generations were, however, smaller than the first and second. This want of complete success was certainly due in part, if not altogether, to the difficulty of obtaining a regular supply of animals of suitable age, and to the frequency with which the mother rats killed their young.

When a tumour which has been kept growing in a succession of rats for as long as 46 days is again inoculated into mice it grows as vigorously as the tumours which have never been grown in rats. In other words, it is not modified by change of "soil"; the tumour cell is killed outright more readily than it is changed in character. It does not seem possible to cause the tumour to adapt itself to its new species of host so completely as to lose its capacity to grow in the species in which it arose. This practically amounts to saying that a mouse tumour cannot be converted into a rat tumour any more than a mouse can be converted into a rat. In the experiments in which the attempt was made to grow sarcoma (37) in half-grown rats, the tumour failed at the fourth inoculation, the growths becoming progressively smaller. Altogether the tumour was kept growing for 22 days in three generations.

One of the most interesting of Murphy's discoveries in his work with chick embryos is that the inoculation of a fragment of adult fowl spleen or bone marrow prevents the growth of a rat tumour inoculated simultaneously.[6] The addition of spleen or, to a less extent, of bone marrow enables the chick to resist the growth of the tumour cells. These experiments have been repeated with young rats, using spleen taken from a healthy adult rat. Half a litter of eight new-born rats were inoculated in the right axilla with 0·01 c.c. of an emulsion of mouse carcinoma (63); the remainder were inoculated in the left axilla with the same dose of the same tumour and in the right axilla with 0·01 c.c. of rat spleen. The growths obtained

are represented in Chart D, which shows that the spleen has had no effect on the growth of the tumour. The same experiment was carried out with sarcoma (37) and with carcinoma (206), with similar results. It is probable, therefore, that the

CHART D.



New-born rats inoculated with mouse carcinoma (63) and with spleen obtained from an adult rat; doses 0·01 c.c. (*a*) Animals 1 to 4 received tumour in right axilla. (*b*) Animals 5 to 8 received tumour in left axilla and 0·01 c.c. of spleen in right axilla. It will be noted that no difference is observable in the amount of growth in (*a*) and (*b*). Scale as in Chart C.

mechanism of the production of resistance in chicks to a tumour of a foreign species is different from that in young rats. The change from the new-born to the adult condition must take place fairly rapidly, as young rats a few days old already inhibit to a marked degree the growth of most inoculated mouse tumours.

The temporary growth and ultimate disappearance of mouse tumours in new-born rats do not differ essentially from the results of similar inoculations into adult rats. In them also temporary growth, often only recognisable on microscopic examination, precedes the rapidly ensuing arrest of growth and absorption of the foreign tumour cells. Bashford and Russell[8] have shown that this disappearance follows on the development of an active immunity. If baby rats in which tumours of considerable size have developed and been absorbed be re-inoculated with fresh mouse tumour, it is seen that they are immune in the same way, and the sequence in the new-born rat only differs from that in the adult in the relative proportions of the two stages of growth and absorption. There is no valid reason to assume a different cause for the ultimate disappearance in the two cases, and the actively immune condition which has been demonstrated in both suffices to account for it completely. The disproportionate amount of growth obtainable in new-born rats is in all probability due to a failure, or rather delay, in the process of active immunisation. It must not be forgotten, however, that young animals are in general more suitable for tissue transplantation than old. Propagation in series is in consequence possible in very young animals by taking advantage of the rapid growth that occurs in the period which precedes the development of inimical substances. The success which has attended the attempts of several investigators to cultivate mammalian tumours and normal tissues *in vitro* in the plasma of alien species also points in the same direction, as in these circumstances the cellular elements responsible for the development of immunity are absent.

The results of these experiments are mainly of importance because of the analogy they present to the much more obscure process of immunity production by mice and rats to transplantable tumours arising in individuals of their respective species. This analogy is not very close, but it is instructive to note that Russell[7] has shown that those propagable tumours which grow progressively do not produce

resistance to a subsequent inoculation. In alien species, however, even the strains which grow without inducing resistance in their own species exhibit only temporary growth and induce active immunity. The experiments will be continued, and the discussion of their bearing on this and other aspects of the cancer problem is reserved for a later date.

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Printed at The Lancet Office,
423 Strand, London, W.C.