EARLY STAGES OF THE TRANSPLANTATION OF THE EHRLICH-PUTNOKY TUMOUR INTO MICE AND RATS

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(From the Imperial Cancer Research Fund)

In 1930 Putnoky, from the laboratory of Professor von Balogh, reported the successful propagation of a mouse tumour in rats. The tumour used was obtained from the Institute of General Pathology, Vienna University, and was described as a strain of the Ehrlich mouse carcinoma. Transplantation was effected by introducing pieces of tumour of the size of a lentil into the subcutaneous tissues of the back through an incision. The rats weighed at least 80 gm. After growing progressively in the rat for about ten days, the tumour regressed or became completely necrotic. It was transplanted from rat to rat every eight to ten days and at the time of publication was in its 28th passage in rats. It had been growing in rats continuously for four years and eight months when Professor von Balogh kindly sent two of these animals bearing the tumour to the Imperial Cancer Research Fund.

As this is the first reported instance of the continued propagation of a mammalian tumour in a foreign species, it was considered worth while to study the early stages of the tumour's growth in the rat and mouse. Furthermore, as histological preparations of the Ehrlich-Putnoky tumour differ widely from preparations of two strains of the Ehrlich mouse carcinoma which were kindly supplied by Dr. Santesson of the Radiumhemmet, Stockholm, it was thought that a study of the early stages of the former tumour might help to explain its anomalous appearance.

The Ehrlich-Putnoky tumour (Fig. 1) consists of large spindle-shaped or polygonal cells with very little intercellular substance. There is no alveolar arrangement and very little stroma. The first of Dr. Santesson's preparations is of the strain kept at the Radiumhemmet and was originally obtained from Professor O. Teutschlaender, Heidelberg. The second (Fig. 2) is of a strain kept at the Rockefeller Institute, New York, and originally obtained from Dr. A. Fischer, Copenhagen. Both are preparations of an alveolar carcinoma with abundant stroma and well formed blood-vessels.

MATERIAL AND METHODS

A series of 12 mice and 12 young rats were inoculated subcutaneously with two small pieces of the Ehrlich-Putnoky tumour—on either side of the ventral mid-line—by means of a fine trocar. The tumour material used was taken from a mouse which had been inoculated from one of the tumours sent by Professor von Balogh. At four, eight, twenty, and twenty-seven hours, and at two, three, and four days a mouse and a rat were killed and the grafts, along with a small amount of normal tissue, were cut out with curved scissors.
FIG. 1. A SIX-DAY EHRICH-PUTNOKY TUMOUR IN THE MOUSE

The tumour consists of spindle-shaped or polygonal cells with very little intercellular substance. There is no alveolar arrangement. Azo-carmine and anilin blue. × 175.

FIG. 2. EHRICH MOUSE CARCINOMA

This preparation was supplied by Dr. Santesson of the Radiumhemmet, Stockholm, and is of a strain kept at the Rockefeller Institute, New York. It is an alveolar carcinoma with abundant stroma and well formed blood vessels. Haematoxylin and eosin. × 230.
Fig. 3. Twenty-seven-hour graft of the Ehrlich-Putnoky tumour in the mouse

There is extensive invasion of the fibrinous exudate surrounding the graft. The tumour cells are easily distinguished from the normal connective-tissue cells by their size and by their distinctive nuclei. Heidenhain's iron haematoxylin. × 310.

Fig. 4. Twenty-seven-hour graft of the Ehrlich-Putnoky tumour in the rat

Although the graft is proliferating, as shown by one mitotic figure, there is no outgrowth of individual tumour cells into the tissues of the host. There is a greater number of mononuclear cells in the tissues surrounding the graft than in the mouse. Heidenhain's iron haematoxylin. × 310. (Figs. 3 and 4 drawn by A. V. Cobbett)
The two grafts from each animal were fixed in Flemming's solution and Zenker-formol respectively. After two hours' fixation the Flemming material was washed overnight, rapidly dehydrated, and embedded in paraffin. The Zenker-formol material was brought through in the usual way. In every case the whole graft was cut in series. The Flemming material was stained by Heidenhain's iron haematoxylin and the Zenker-formol material by various methods.

**Fig. 5. Periphery of a Two-day Graft of the Ehrlich-Putnoky Tumour in the Rat**

The tumour cells have abundant cytoplasm and vesicular nuclei with large nucleoli. A blood vessel has been invaded and there is phagocytosis of blood cells by the tumour cells. Two telophases are shown. Heidenhain's iron haematoxylin. × 800. (Drawn by A. V. Cobbett)

**Results**

The results obtained can be described by a review of Figs. 3 to 7, which illustrate all the main features of this tumour's growth in the mouse and rat.

**Fig. 3:** Twenty-seven hours after transplantation into the mouse the Ehrlich-Putnoky tumour has already extensively invaded the surrounding fibrinous exudate. The numerous polymorphonuclear leukocytes in and around the graft constitute the usual reaction to transplanted tumour fragments at this stage. The tumour cells with their large nuclei are easily distinguished from the much smaller connective-tissue cells of the host.

**Fig. 4:** Twenty-seven hours after transplantation into the rat there is no outgrowth of individual tumour cells into the tissues of the host, although the cells in the graft are proliferating, as shown by one mitotic figure. As in the mouse, there are numerous polymorphonuclear leukocytes but there is, in addition, a great increase in the number of mononuclear cells. The tumour
cells are distinct from the normal cells and there is no transformation of rat cells to tumour cells.

Fig. 5: This figure is from the periphery of a graft in the rat two days after transplantation. The tumour cells are polygonal or spindle-shaped, and the nuclei are vesicular with coarse, granular chromatin and large nucleoli. Two telophases show the ability of the tumour to proliferate in the foreign host. A blood vessel has been invaded and an interesting feature is shown in the phagocytosis of red blood cells by tumour cells.

Fig. 6: At four days in the mouse there is a massive outgrowth of tumour cells into the fibrinous exudate with very little reaction on the part of the host.

The connective tissue of the host is surrounded by the tumour but does not build a formed stroma.

Fig. 7: This figure shows the border of a four-day graft in the rat and its relation to the tissues of the host. Surrounding the tumour are large numbers of mononuclear cells and an increase of the connective-tissue cells. The graft itself is invaded by numerous mononuclear cells, and many of the tumour cells are necrotic. The sharp distinction between tumour cells and host cells and the absence of tumour cells outside the graft provide conclusive evidence that there is no sarcomatous transformation of the host tissues. Such a picture is characteristic of the reaction of an animal to the transplantation of a tumour to which it is resistant, in this case a tumour from a foreign species. The Ehrlich-Putnoky tumour, therefore, when transplanted in the rat, grows
by a proliferation of its own cells and continues to grow only until the de-
fensive powers of the host get the upper hand.

To avoid the criticism that the results were vitiated by the fact that the con-
tinuous cultivation of the Ehrlich-Putnoky tumour in rats had been in-
terrupted by transplantation into mice for one generation, a further series of early stages was studied. Dr. J. Putnoky kindly supplied a rat bearing an Ehrlich-Putnoky tumour which had been growing continuously in rats for six years and six months. The rat-grown tumour was transplanted into a series of rats and mice and the early stages studied as described above. The re-
sults were in all respects identical with those after transplantation with the mouse-grown tumour. In Figs. 8 and 9 the growth of rat-cultivated Ehrlich-
Putnoky tumour in the mouse and rat is compared three days after inocula-
tion. At three days in the mouse (Fig. 8) the graft, which was introduced under the panniculus carnosus, has gone through the muscle and is infiltrating the dermis. This type of early outgrowth and invasion is characteristic of a rapidly growing sarcoma. A carcinoma such as the Ehrlich mouse carcinoma (Fig. 2) has not yet received from the host the supporting stroma which is necessary for its progressive growth. At three days in the rat (Fig. 8) the graft, although proliferating, is sharply delimited by the muscle, showing that it does not behave like a malignant tumour in the rat. At four days in the rat (Fig. 10) the graft consists of scattered tumour cells and is massively infiltrated with mononuclear cells. In spite of its long uninterrupted cultivation in rats, the rat reacts to the Ehrlich-Putnoky tumour as to a foreign tumour. The comparison between the growth of the tumour in the mouse and in the rat

FIG. 7. FOUR-DAY GRAFT OF THE EHRlich-PUTNOKY TUMOUR IN THE RAT
Numerous mononuclear cells are surrounding and invading the graft. Most of the tumour cells are already necrotic. Heidenhain's iron haematoxylin. X 195.
FIG. 8. THREE-DAY GRAFT FROM A RAT-GROWN EHRLICH-PUTNOKY TUMOUR GROWING IN THE MOUSE

The graft, which was introduced under the panniculus carnosus has gone through the muscle and is infiltrating the dermis. Heidenhain’s iron haematoxylin. × 100.

FIG. 9. THREE-DAY GRAFT FROM A RAT-GROWN EHRLICH-PUTNOKY TUMOUR GROWING IN THE RAT

The graft, though proliferating, is sharply delimited by the muscle, showing that it does not behave like a malignant tumour in the rat. Heidenhain’s iron haematoxylin. × 100.
demonstrates that, in spite of its six and a half year’s existence in a strange environment, it still retains the characters of a mouse tumour.

**DISCUSSION**

From the study of the early stages of the Ehrlich-Putnoky tumour it may be concluded that it is not an alveolar carcinoma and therefore differs fundamentally from the original Ehrlich mouse carcinoma. Various possibilities present themselves as an explanation. This tumour may be a very anaplastic strain of the Ehrlich carcinoma. There may have been a sarcomatisation of the stroma with disappearance of the original carcinoma, as was described by Haaland in 1908, in mouse tumour 37 of the Imperial Cancer Research Fund. Another possibility is the accidental substitution of another tumour, transplantable or spontaneous. It is important to realise that Putnoky’s results are applicable to the Ehrlich-Putnoky tumour and not to the Ehrlich mouse carcinoma as such. In this connection it is significant that Bullock (1915) got much more growth in rats with mouse sarcoma 37 (polymorph strain) than with the mouse carcinomas 63 and 206.

For a full review of the many publications on heterologous transplantation, reference may be made to the excellent monograph by Kerguntul (1933). Ehrlich (1907) showed that a mouse tumour could grow progressively in the rat for a period of eight to ten days, after which it invariably regressed. By zig-zag transplantation between rat and mouse he was able to cultivate a mouse.
tumour through fourteen passages. The tumour lost none of its growth energy and was in no way altered by its sojourn in the rat.

Bullock (1915) succeeded in cultivating mouse sarcoma 37 (polymorph strain) in new-born rats for five passages over a period of forty-nine days. He was prevented from carrying this further by the difficulty of obtaining a regular supply of new-born rats and by the frequency with which the mother rats killed their young after they had been interfered with. He was not so successful with half-grown rats, where he failed at the fourth passage. In these experiments Bullock used minute doses of tumour as employed in routine transplantation.

Putnoky's results are essentially the same as those of Bullock. He owes his success in continuing the growth in series to two things. In the first instance he has been fortunate in the choice of a tumour which grows extremely rapidly and is independent of stroma production by the host. Secondly, he obtains a much more extensive proliferation by the introduction of large grafts.

The only other recorded example of continued propagation of a tumour in a foreign host is that of the Fujinami fowl myxosarcoma, which was transplanted through forty generations in ducks (Fujinami and Hatano, 1929). Gye (1931) confirmed this and further demonstrated that a cell-free filtrate of a fowl-grown Fujinami myxosarcoma could produce a Fujinami myxosarcoma in a duckling, thus proving that the successful heterotransplantation of this tumour depends on the ability of the fowl tumour agent to infect duck cells. Purdy came to the same conclusion in a further analysis by two types of experiment. In the first, ducklings which had received an injection of minced fowl embryo resisted to some extent the growth of minced fowl-grown Fujinami myxosarcoma, whereas they completely resisted the growth of Rous sarcoma. Purdy concluded that the initial proliferation of the implanted cells had been prevented and that the subsequent Fujinami tumour was a result of the action of the infective agent (Purdy, 1933a). In the second type of experiment, filtrate obtained from a Fujinami tumour growing in the duck was partially neutralised by an anti-duck serum and not at all by an anti-fowl serum. Thus the tumour agent had acquired duck characters and was therefore derived from duck cells (Purdy, 1933b). In short, when the Fujinami myxosarcoma is transferred from fowls to ducks it becomes a duck tumour in that it is composed of duck cells. On the other hand, the propagation of the Ehrlich-Putnoky tumour in rats depends on the proliferation of the implanted mouse tumour cells.

This cultivation in series of a mouse tumour in rats is quite different from the propagation of the same tumour in mice. In the individual mouse the growth is progressive and continues to be progressive until the death of the animal. In the individual rat there is a temporary survival of the tumour with sufficient proliferation to give measurable growth provided the initial graft is big enough. There is no sarcomatisation of the rat tissues and in no sense can the animal be said to be suffering from cancer. Such a growth can be of no value in the study of cancer, particularly in therapeutic experiments.

That this is not appreciated may be demonstrated by reference to two publications by Ottenstein and Pastinszky (1935) entitled "Therapeutic re-
searches with ferments in experimental transplantable rat tumours” (Ottenstein and Pastinszky) and “On the lead therapy of cancer and the influence of some colloidal metals on the growth of experimental transplantable rat tumours” (Pastinszky and Ottenstein). They state that they have employed the Ehrlich-Putnoky rat tumour strain throughout their experiments, but in neither publication do they mention that it is a heterologous tumour.

**Summary**

The Ehrlich-Putnoky tumour is a sarcoma or a very anaplastic strain of the Ehrlich carcinoma, which grows with great rapidity.

Its rapidity of growth probably explains the ease with which it lends itself to heteroplastic transplantation.

Its growth in the rat is a temporary proliferation and is unaccompanied by sarcomatisation of the normal connective tissue.

Heterologous transplantation is of little importance in the study of cancer. General conclusions based on results of experiments where heterologous tumours have been used are valueless.

**References**


Purdy, W. J.: Brit. J. Exper. Path. 14: 9, 1933. (a)
