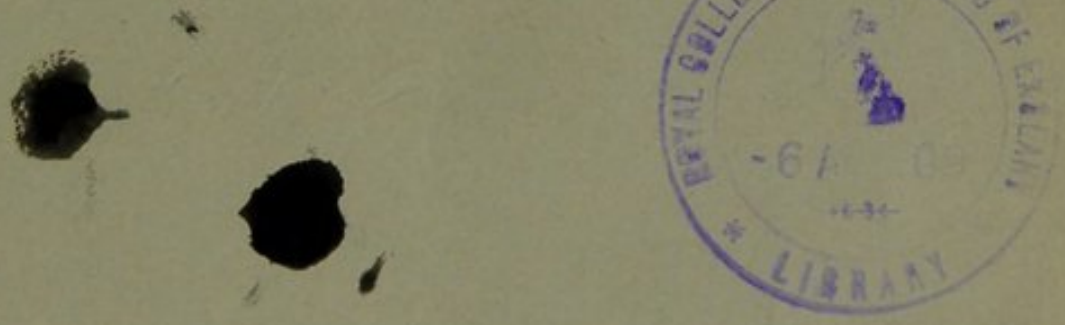
**Proceedings of the Pathological Society of Great Britain and Ireland.**

# PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN AND IRELAND.

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# THE INDUCTION OF SPECIFIC RESISTANCE AND OF GENERAL ENHANCED SUSCEPTIBILITY TO INOCULATION OF CARCINOMA AND SARCOMA IN RATS AND MICE.

By E. F. Bashford, J. A. Murray, and M. Haaland.

The paper dealt in the first place with investigations with a transplantable squamous-celled carcinoma. The importance of the methods employed in inoculation and in recording the results was emphasised. Illustrations were given of the influence of variations in the dose of tumour material, in the interval between inoculations, and in the age of the animal. Particular

attention was directed to the alternation of positive and negative phases in the growth of propagated tumours. That immunity or resistance can really be induced was shown by the results of interposing an inoculation of normal tissue or of spontaneous tumour between a primary successful inoculation and a subsequent one. It was also shown that, while a large dose (0·1 c.c.) of tumour tissue may be followed by transitory growth and absorption, a small dose (0·05 c.c.) was followed by progressive growth. This fact demonstrated that one had to deal with mice which were not naturally resistant but had been rendered so artificially.

From large series of experiments the following conclusions were drawn as to the nature of the protection of one tumour against another: The resistance induced by the absorption of carcinoma is effective against the tumour primarily inoculated, and to a much higher degree than against other carcinomata. It is often absolute for the same tumour. No evidence has as yet been obtained that the absorption of carcinoma tissue increases the resistance to sarcoma to an extent surpassing that following the absorption of normal tissue. As regards the absorption of sarcoma, it was remarkable that the mice were thereby protected against carcinoma to a very high degree. These results were in contradiction to those of Ehrlich, who found a "pan-immunity," *i.e.* the protection between carcinoma and sarcoma was mutual and of equal degree.

Of the normal tissues of the mouse, skin alone was able to induce a nearly absolute degree of protection against squamous-celled carcinoma. The rest of the tissues, either collectively or individually (*e.g.* mamma, reproductive tissue), were devoid of this power: the relation of cancerous with gametic tissue was less than with the somatic tissues. The fact that the rest of an embryo protected against squamous-celled carcinoma to a much less extent than did its skin was incompatible with the view that various ferments were responsible for the protection induced. Anomalous results obtained gave evidence that growth could not only be hindered but also favoured by previous treatment with normal tissues. Whereas protection was only conferred by tumours or tissues of the same species, growth could be favoured by previous treatment with tissues of strange species. By heating skin previous to inoculation the protection was converted into a condition favouring growth. It could no longer be doubted that the development of a tumour from a very circumscribed area might be determined by constitutional conditions favourable to growth. The methods employed had taken the investigations beyond the regions reached by histological methods alone; they constituted really a new branch of experimental biology, enabling one to analyse the relations between the tissues of individuals of the same species.

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## THE PROCESSES AT THE SITE OF INOCULATION IN NORMAL MICE AND IN MICE RESISTANT TO CARCINOMA.

By B. R. G. Russell.

The experiments were mostly done with a slowly growing adeno-carcinoma of mouse mamma. In normal mice the introduced stroma degenerates and the new stroma for the developing acini is developed from the tissues of the new host. In mice resistant to the adeno-carcinoma there is no such development of new stroma, and the graft becomes converted into a small cyst lined with a single layer of cubical epithelium. The conclusion arrived at is that in resistant mice the cancer cells were so acted upon as to prevent them calling forth the stroma reaction on the part of the connective-tissue cells.

## THE CLINICAL BEHAVIOUR OF SPONTANEOUS TUMOURS IN MICE.

By J. A. Murray.

The spontaneous new growths of the mammary region of the mouse pursue the ordinary course of malignant growths met with in other animals and in man. The apparent encapsulation is very incomplete as seen by histological examination, and the results of extirpation confirm this. Local recurrence occurs in more than half the cases in which total surgical removal has been attempted. The interval which elapses between the operation and the recurrence varies from two or three weeks to several months. More remarkable are cases in which, without local recurrence, metastases are found in the lungs after a long interval. There is no clear evidence that operative interference increases the malignancy of the tumours. It is probable that some of the cases described as multiple tumours are in reality metastases.

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## A TRANSPLANTABLE SQUAMOUS-CELLED CARCINOMA.

By J. A. Murray and M. Haaland.

The growth in question occurred in the left axillary region of an old female mouse. The primary growth was strongly keratinised in its central parts, while the periphery or more newly formed portions were mainly alveolar. A metastasis found in the lungs of the primary animal after death showed large areas of perfect keratinisation in the centre of the nodule; the periphery was alveolar as in the primary growth. The transplanted tumours, obtained after great initial difficulties, were at first purely alveolar carcinoma. Keratinisation again appeared in the eighth generation, so that large areas of the tumours consisted of horny masses. This condition persisted for two more generations, and then disappeared for several generations extending over a period of nearly three months. It has again appeared in some of the later tumours. The conditions governing the differentiation are not yet understood; an inherent periodicity in the life of the parenchyma cells seems to be the most satisfactory assumption to account for the facts at present.

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## DEVELOPMENT OF A SPINDLE-CELLED SARCOMA DURING PROPAGATION OF AN ADENO-CARCINOMA OF A MOUSE.

By M. Haaland.

When a small piece of a carcinoma of the mouse is implanted into normal animals the parenchyma continues to grow (Jensen), the blood vessels and stroma degenerate, and are supplied afresh by the new host reacting to the chemiotactic influence proceeding from the parenchyma cells (Bashford, Murray, and Cramer). The connective tissue reactions thus elicited is specific for each form of malignant new growth, and without it a "graft" does not grow into a tumour. The parenchyma elements alone possess the power of continued growth in normal cases of successful transplantation, the degeneration of the transplanted stroma being invariable.

Attempts to induce cancer experimentally by applying chemical, physical, or bacterial irritants, or to obtain tumour growth by transplanting normal embryonic and adult tissues, have, without exception, failed. In the course of the continued propagation of two alveolar carcinomata, Ehrlich and Apolant

observed that an alteration in the character of the tumours had taken place. A mixed tumour was suddenly observed, and the alteration progressed till only sarcoma remained. The importance of this observation is very great. The criticism (Schlagenhaufer, von Hansemann) that the tumours had contained sarcomatous elements from the outset could not be met entirely, owing to the unexpected appearance of the change and the lacunæ in the material available for investigation afterwards. As Bashford pointed out, it was not demonstrated that the stroma cells previously normal had suddenly or gradually changed their character in the process of propagation since the processes at the site of inoculation had not been followed step by step either for the primary or for the transplanted tumours.

The processes at the site of inoculation of "grafts" of primary tumours have been systematically studied for all spontaneous tumours observed in the Imperial Cancer Laboratory, as well as at repeated intervals when propagation has succeeded. A large slice is also preserved from each tumour transplanted.

In the course of propagation one tumour, an adeno-carcinoma, has shown a remarkable phenomenon. There was no doubt whatsoever that at the primary and several subsequent transplantations the stroma degenerated. At the eighth transplantation the cellular elements of the stroma were of a different character, and their subsequent behaviour showed that they also were thenceforward endowed with powers of independent growth when transplanted into new hosts.

A series of slides were shown of the primary adeno-carcinoma mammæ, and of its behaviour during propagation. Up to the seventh transplantation the character remained unaltered, the parenchyma being at times more adenomatous, at other times more alveolar. The stroma remained very sparse. The sudden change in the character of the stroma in four out of seven tumours of the seventh transplantation was shown. The connective-tissue elements had become more abundant, and contained large spindle cells. The change was general in three and localised in one of these tumours. The mother tumour used to yield the seventh transplantation showed no deviation from the normal. In succeeding transplantations the change progressed and a previously unrecorded phenomenon was observed, the scattered alveoli of the parenchyma being often surrounded by "halos" of large polymorphous cells, easily distinguishable from the epithelial cells, and of connective-tissue origin.

The stroma of all transplantable carcinomata may become more abundant or more cellular, *e.g.*, in spontaneous healing, etc., but such stroma has not been transplanted. Subsequent, however, to the change above described in the connective tissue of this tumour, it behaved differently when transplanted. The connective tissues no longer degenerated entirely, but the spindle cells continued to grow. A slide was shown illustrating the continuous growth of the connective tissues side by side with the epithelium, numerous mitoses being present in the spindle cells twenty-four hours after implantation of the graft. Another slide illustrated the degeneration of the stroma in other parallel series of the same tumour in which the stroma still retained its original properties.

The observations were confirmed later in another distinct strain of the same tumour in the case of one out of three tumours of the eighth transplantation,—the descendants of the two sister tumours had up till the present maintained their purely carcinomatous character without alteration in the stroma. From the exceptional tumour seventeen daughter tumours were obtained, and thirteen of them were also transplanted. The change in the connective tissues progressed in them all. In over 400 tumours in other parallel strains of this adeno-carcinoma the characters of the primary growth have been maintained up to the present time.

The conclusions drawn provisionally were that the primary tumour was

not a mixed tumour; the changed character of the connective-tissue elements was not of a granulomatous nature, but was a true spindle-celled sarcoma, capable of transplantation and of giving rise to metastases; the epithelial cells had not been transformed into spindle cells. A malignant new growth had been produced *de novo* and experimentally from normal connective tissues. As to the manner of production, the author stated no definite explanation could yet be advanced. Possibly the character of the parenchyma of this particular tumour was of more importance than the constitutional qualities of the hosts whose connective tissues had acquired the properties of malignant new growths. The phenomenon had been observed in only one of forty transplantable carcinomata, but had recurred in that particular tumour, while no similar change had been observed in the thousands of mice inoculated with other carcinomata. The number of animals in which the change had been suddenly observed spoke in favour of that view. As previously pointed out by Bashford, there was no evidence that the connective tissues had acquired sarcomatous properties in consequence of the repeated passage from one mouse to another of the descendants of the stroma cells of the primary growth. There was no evidence that a virus had been transferred from the parenchyma to the connective-tissue cells. Were this the case the protection conferred by the carcinomatous and sarcomatous elements would be due to a common virus, and this protection one would expect to be mutual, which was not the case. As a matter of fact, the sarcomatous element had been freed from carcinoma by passage through mice highly immune to the carcinomatous component of the "mixed" tumour stage. (*Vide* also *Berl. klin. Wochnschr.*, 1907, September.)

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## THE OCCURRENCE OF GLYCOGEN IN MOUSE TUMOURS.

By M. Haaland.

The hypothesis has been advanced by Brault and others that the presence of glycogen in malignant new growths is proportionate to their rate of growth (malignancy), and that the determination of the amount of glycogen permits of conclusions as to prognosis. In transplantable mouse tumours the rapidity of growth exceeds anything met with in the vertebrates, and presents a biological problem of which the importance is not restricted to its bearing upon cancer.

The presence of glycogen was sought for by Best's carmine method controlled by various iodine methods. The tumours investigated were (1) several spontaneous adeno-carcinomata of the mamma of the mouse; (2) numerous transplanted tumours (both adenomatous and solid), especially strains 27, 37, 46, 50, 39, and Jensen's tumour; (3) a transplantable squamous-celled carcinoma; (4) a mixed tumour; (5) a transplantable sarcoma of the mouse and one of the rat; (6) Flexner's carcinoma of the rat.

In the parenchyma of those tumours distinct glycogen staining was found only in the case of the squamous-celled carcinoma, and in the site corresponding to its occurrence in healthy skin. In the others glycogen was found in the surrounding fatty tissue in the stroma cells, or in leucocytes, nothing more than a faint diffuse reaction being obtained in the parenchyma cells. The occurrence of fat was also recorded by means of sudan, etc., without any relation to the occurrence of glycogen being elicited.

The conclusion was drawn that there appears to be no relation between the amount of glycogen in the tumour cells and their rate of growth. Experimental study therefore confirmed the conclusion arrived at by Lubarsch and Gierke for human tumours. So far as the occurrence of glycogen is concerned, the tumour tissue presented the same features as the corresponding normal tissue, namely, skin.