The mast cells described by Ehrlich (6) are characterized by granules showing a so-called true metachromasia. Using as a basis Lison's statement (22) that this color reaction is typical of macromolecular sulphuric ester compounds and Jorpes' (17) demonstration that heparin is a mucoitin ester sulphuric acid showing a distinct metachromasia, Holmgren and Wilander (11) proved the mast cell granules to consist of a material with the same properties as heparin. These authors were able to isolate 22 mgm. of active heparin from 10 gm. of mast-cell-rich capsule of cow liver, whereas preparations of mast-cell-poor capsule of lamb liver proved to be inactive. Jorpes, Holmgren and Wilander (19) also confirmed the conception of mast cells as bearers of mucoitin ester sulphuric acid (heparin). Later this was corroborated by Hirth (10). Wilander (33) in a more comprehensive treatise on the nature of heparin demonstrated the mast cells to be the bearers of heparin. Mast cells are found exclusively in connective tissue, in rather variable amounts within the different parts of the body. Ehrlich had already emphasized that these cells occur chiefly in the vicinity of blood vessels. For bibliographical notes pertaining to mast cells, see Lehner (20), Holmgren and Wilander (11) and Michels (25).

The pathology of mast cells seems still to be only partially recognized. Maximow (24) states that mast cells change their appearance during inflammation. Some are said to burst, and their granules are found in the tissues. Maximow also emphasizes that the new tissue is free from mast cells. Nakashima agrees with Maximow and stresses the fact that during the degeneration of mast cells the granules lose their metachromasia. In addition, Ernst (8) describes morphologic changes of mast cells during the first hours of the inflammatory process.

The appearance of mast cells in tumors of various kinds has been dealt with by many authors. Sylvén (31) described the appearance of mast cells in sarcoma of connective tissue origin. He emphasized that the largest number of these cells has "always been demonstrated within the peripheral parts of the tumors, where the infiltrative destructive growth and disintegration of surrounding normal tissues takes place." On the other hand, mast cells occur only occasionally in the central parts of the tumor. Sylvén (31) has reviewed the literature on mast cells in mesenchymal tumors.

The fact that several authors (1, 3-5, 9, 21, 26, 28, 29, 32) have pointed out the large numbers of mast cells to be found in the skin of mice with tar cancer is of considerable interest for our work. Mast cells will sometimes even form proper nevi (3, 4, 9, 29). Borrel, Boez and de Coulon, (3) who were of the opinion that cancer is caused by a virus, thought that the mast cell reaction "opened the door to all kinds of infections and thus favoured the development of cancer," whereas Cramer and Simpson (4) believed the accumulation of mast cells to be a defensive process directed against the development of cutaneous cancer.

Cramer and Simpson (4) carried out a thorough investigation of the appearance of mast cells during the development of skin cancer after application of a 0.6 per cent solution of methylcholanthrene in benzene to the dorsal skin of mice. They demonstrated the accumulation of mast cells as a rule to be proportional to the epidermal hyperplasia and considerably to precede the development of malignant growth. In the tumor itself mast cells are scarce but are found in large masses in the immediate neighborhood of the growth, especially where the adjacent epidermis shows advanced hyperplasia. Bloom (2) described the spontaneous appearance of tumor-like masses of mast cells in the skin of older dogs without skin cancer. Cramer and Simpson (4) pointed out that this observation is of interest, as skin cancer is relatively common in dogs.

With regard to human pathology, the appearance of large masses of mast cells in lesions of a fairly rare skin disease, e.g. urticaria pigmentosa, should be mentioned. No relationship of this disease to skin cancer is known.

Finally the fact may be recalled that human myeloid leukemia is characterized by an increased number of blood-mast cells, as described by Holmgren and Wohlfart (14). The relative percentage of blood-mast cells may sometimes rise to about
in 4 cases by injection of benzpyrene. All 3 produced in white rats was studied. In 27 cases previously into the back by means of a trocar. These varying type. In the transplantation experiments however the result was somewhat less satisfactory proved histologically to be a sarcoma, although of methylcholanthrene, in 16 cases by implantation of pieces of methylcholanthrene-cholesterol, and in 4 cases by injection of benzpyrene. All 3 methods resulted in sarcomas of varying types. The majority of the tumors were of polymorpho-cellular or fibroblastic character. Frequently the type of tumor changed in different portions. In a small number of cases lipomyxomatous and rhabdomyosarcomatous types were observed. When stained with toluidine blue, cells containing metachromatic granules, i.e. mast cells, appeared in varying number in all tumors, in the tumor tissue as well as in the capsule. While mast cells occurred rather infrequently or were almost lacking in the polymorphocellular tumor portions (sometimes there were none in several visual fields), they were usually present in large numbers, and sometimes abundantly in the fibroplastic portions (Fig. 1). In the rather infrequently observed lipomyxomatous portions only occasional mast cells were found, whereas their number was somewhat larger in the rhabdomyosarcomatous portions.

In Figs. 1, 2 and 3 the varying mast cell incidence in fibroblastic sarcoma portions will be seen. Definite interrelation with the intratumoral blood vessels was not noted. Neither did there emerge a connection between the degree of vascularization and the percentage of mast cells. The polymorphocellular sarcomas were, by the way, extraordinarily vascularized.

As a rule there was no morphologic difference between the mast cells within the tumors and those situated in other tissues. The content of granules in these cells varied greatly (Fig. 4). Within the frequently occurring necrotic areas, uninjured mast cells were sometimes seen (Fig. 5). This might be taken to indicate a greater resistance of mast cells than of other cells. If mast cells are amoeboid cells, as Lehner (20) and others assume, one might suppose that they invade secondarily the necrotic portions. However, the fact that mast cells as a rule are regularly scattered over the necrotic areas and generally quite as thickly as in the neighboring portions, fails to support this presumption. Sometimes the mast cells situated within the necrotic areas displayed a varying affinity to the stain, so that the granules stained a dark blue or blue-green with toluidine blue. In the most advanced necroses mast cells have completely disappeared or are noticed only as indistinct, considerably degenerated cells. The mast cells with blue to blue-green granules are also found in the normal tumor tissue, although more sparsely. One might blame deficient staining, yet this cannot obtain, since around the cells in question other cells are found with obviously metachromatic granules. According to unpublished observations of Holmgren, these cells occur also under other similar conditions. They are to be regarded as having undergone certain changes.
Figs. 1-5
Diffuse tissue metachromasia was often noted within the tumors. The same type of diffuse metachromasia has previously been described by Holmgren (13) and others in growing embryonal tissue and was recently found by Holmgren and Rexed (15) in the Büngner bands of regenerating peripheral nerves in rats. Sylvén (31) observed in granulation tissue and in various mesenchymal tumors diffuse metachromasia which he called "free chromotrope substance" and which in his opinion is due to mast cells. This conception is based upon a subjective estimation of the quantity of granules and the number of mast cells in tissue containing much or little free chromotrope substance respectively. Sylvén states that fewer mast cells, poor in granules, are present in the former than in the latter. It is of interest to note that Quensel (27) found in cancer a "mucoid" which stains similarly to mast cells, wherefore he presumes it in both cases to be the same substance.

In this investigation no very close attention was paid to the diffuse tissue metachromasia. As for Sylvén's theory, the fact should be stressed that no obvious connection, whatever, between diffuse tissue metachromasia and mast cells content emerged in our material. One could, for example find quite often that areas with strong diffuse metachromasia also contained a large amount of mast cells. These cells, with variable content of granules appear also in parts without diffuse metachromasia.

The enormous number of mast cells that we found in some tumors raises the question of the histogenesis of mast cells. One might ask oneself whether these cells invaded from adjacent structures or originated from the stroma of the tumor tissue. The experimental sarcomas may grow to an exceedingly large size (in our series we met with tumors weighing over 200 gm.). If these tumors are extremely rich in mast cells, as is quite often the case, the neighboring tissue could be expected to be strikingly poor in mast cells, provided the mast cells invaded the tumor from the vicinity. Examination of the tissues surrounding the tumors, however, always showed a fairly normal mast cell content. In no case did a rough subjective estimation of the mast cell content reveal a difference as compared with normal animals. As already mentioned, the tumor capsules contained a remarkable amount of mast cells both in fibroplastic and polymorphocellular sarcoma. A priori the most likely assumption appears to be that the mast cells develop locally in the connective tissue of tumors. The fact that part of the mast cells contain only a few metachromatic granules might also be taken to suggest such an origin. Under these circumstances one would expect to find evidence of mitosis. However, in spite of systematic investigation in no case were signs found indicating mitosis or amitosis. This agrees with the fact that neither Holmgren (15) or others have found mast cells in a state of mitotic or amitotic division in fetuses of rats, mice or man. The appearance of mast cells with only a few metachromatic granules might naturally indicate that these cells had lost most of their granules, and it would be difficult to exclude this possibility in every case. The conception that the granule-poor cells in tumors rich in mast cells might represent young cells where the granules are developing, is supported by a certain similarity of these cells with mast cells in fetuses. There one also finds that the granules appear first in the peripheral parts of the cells as small, sometimes dust-like particles.

In one experiment macroscopically healthy normal tumor pieces were under aseptic conditions transplanted subcutaneously into 10 to 15 young rats. Nearly one-half of the transplanted pieces started growing. From one of the daughter growths new transplantations were carried out in the same way. Thus we followed a tumor through 4 generations, not including the mother growth. Both the mother growth and the various "generations" appeared histologically to be fibrosarcomas. The mother growth contained relatively few mast cells. The first tumor generations presented the same picture as the mother growth and also contained few mast cells. The tumors of the last two generations showed more polymorphous cells and}

**DESCRIPTION OF FIGURES 1 TO 5**

**FIG. 1.**—Fibrosarcoma from white rat, extremely rich in mast cells, and induced by subcutaneous injection of 3, 4-benzpyrene in olive oil. Toluidine blue stain. Mag. × 250 (approx.).

**FIG. 2.**—Sarcoma rich in mast cells from white rat induced by subcutaneous injection of methylcholanthrene in olive oil. Toluidine blue stain. Mag. × 400 (approx.).

**FIG. 3.**—Sarcoma from white rat with fair number of mast cells, induced by subcutaneous injection of methylcholanthrene in olive oil. Toluidine blue stain. Mag. × 250 (approx.).
more necroses than the first generations. Quite naturally it was difficult to determine the exact number of mast cells owing to the varying distribution in the different areas. Therefore, we always examined several portions of the tumors. On the whole we received the impression that with every new generation the picture became more polymorphous, the number of mast cells simultaneously declining. Other transplantation experiments were carried out only through 2 daughter generations. Nothing of interest emerged in addition to these findings.

From our experiments it follows that mast cells consistently occur in experimental sarcomas of white rats, caused by carcinogenic substances, and especially in those of the fibroblastic type, where they sometimes even reach an excessive development. It is of interest that the mast cells accumulate to a certain extent in the tumor capsules encasing the sarcomas, and in the connective tissue beneath the skin hyperplasias and carcinomas artificially produced by Cramer and Simpson (4). The study of tumors by Sylven (31) showed that the number of mast cells in his 21 cases of sarcomas of connective tissue origin varied but that the largest amount was found in the periphery of the tumors where "the infiltrative-destructive growth and disintegration of surrounding normal tissues takes place." According to Sylven, as a rule mast cells are occasionally found in the central parts of the tumors, especially along the vessels. The content of granules in mast cells within the infiltration zone varies widely, and it is not unusual to find only a few granules.

Of course, it is not possible to explain the cause of the high incidence of mast cells in certain experimental sarcomas. One feels rather inclined to the belief that these cells take part in the reaction of the system against tumor cells. If this is the case, however, various types of sarcoma seem to produce a changing reaction in the system. The question whether the varying mast cell content depends upon the degree of differentiation in the various tumor types or upon other factors, must be left in abeyance. The assertion by Brack (3a) that rapidly-growing tumors in the epidermis and in the alimentary canal should contain a large number of mast cells is of interest. It is possible that the organ or tissue in which the tumor grows has some influence on the amount of mast cells in the tumor. Furthermore, the position is complicated by the fact that in some tumors there are alternately areas rich and poor in mast cells without any other difference in their histological appearance. At any rate, the appearance of masses of mast cells seems to characterize certain experimental tumor types. The established fact that mast cell granules consist of the anticoagulant heparin is probably of significance in explaining their appearance in tumors.

The statement of Cramer and Simpson (4) that fixation in formalin dissolves the mast cell granules in rats, resulting in the appearance of granule-poor mast cells is of interest in this connection. Fixation in alcoholformol has not the same effect. In our cases we used lead acetate in addition to formalin. The former coagulates heparin and preserves the mast cell granules in rabbits where they are extremely soluble in other fixation media. In our specimens fixed with lead acetate, we observed the same pictures as in those treated with formalin. Therefore, it does not seem likely that the mast cell granules, which in rats are very resistant to water, can be dissolved (12). The inference of Cramer and Simpson (4), that in rats mast cell granules occur in a water-soluble and an insoluble form, does not seem to be satisfactorily established. Furthermore, the authors state that a powerful mast cell reaction develops after treatment with methylcholanthrene and that "certain groups of mast cells show a strong golden-brown fluorescence." According to others and from our own experience, normal mast cells possess no auto-fluorescence. Since methylcholanthrene, which was applied to the skin, has an auto-fluorescence, it appears more likely that the fluorescent cells observed by Cramer and Simpson (4) were macrophages loaded with substance. Admittedly, the fluorescence of methylcholanthrene is bluish, but we do not know whether this color is changed by the fixation. This possibility was disregarded by Cramer and Simpson (4). In addition, F. Sjostrand (30) found the macrophages to emit a pronounced fluorescence, a fact that should be borne in mind in this connection. This fact should be considered before accepting the statement of Cramer and Simpson as to the auto-fluorescence of mast cells under the conditions described by them.

SUMMARY

Experimental sarcomas in white rats produced by carcinogenic substances regularly contain mast cells. In such sarcomas of fibroplastic type the development of mast cells can attain an extreme degree.

The mast cells present in experimental sarcomas seem to develop locally in the connective tissue of the tumors, and this suggests that they take part in the reaction of the system against the tumor cells. Within the necrotic tumor areas the mast cells
are frequently well preserved, a fact that seems to point to these cells possessing a greater power of resistance than the tumor cells.

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Mast Cells in Experimental Rat Sarcomas

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