from National Cancer Institute, U.S.P.H.S.

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Transplantable Mastocytoma in the Mouse Containing Histamine, Heparin, 5-Hydroxytryptamine.* (23375)

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Mast cells are among the current problem cells of the body. Several vital functions have been attributed to them but the relation of any functions to the mast cells has not been firmly established. They are said to be the major, if not the sole, source of heparin (1); so they may be involved in the clotting mechanism. Their relation to connective tissue and their high content of heparin suggest that they may be involved in the elaboration of the mucopolysaccharides of the collagenous ground substance. They are a rich source of histamine(2) and may play a part in processes of inflammation and hypersensitivity. It has been reported that they contain 5-hydroxytryptamine (serotonin, 5-HT)(3), a naturally occurring amine with pharmacological actions on smooth muscle. The distribution of mast cells in certain locations and in certain tumors apparently follows a definite pattern(4), the significance of which is not clear.

Tumors composed of mast cells are well known in animals; none has been reported in man. The most studied is that occurring occasionally in dogs. It was first described by Bloom(5) and its high heparin content established by Oliver *et al.*(6). Deringer and Dunn(7) have described a mouse mastocytoma. Transplantation of a mastocytoma, apparently carried out simultaneously with that described here, has just been reported by Dunn and Potter(8). A survey of the relevant literature is beyond the scope of this report.

Origin. The tumor was found in a 500-day-old mouse of the LAF_1 strain, that had been irradiated at approximately 7 weeks of age with 475r over the entire body. The left adrenal of the animal was removed 5 weeks following irradiation. This was one of a large

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series in which groups of approximately 100 animals were subjected to similar though smaller doses of total or partial body irradiation with or without removal of adrenals or gonads. The data so far do not indicate any relationship between the treatment, including irradiation, and the mast cell tumor. However, as a thorough histologic study of the various neoplasms occurring in this series (over 2000 mice) has not yet been made, the question remains whether or not mastocytomas are induced at a low rate by the procedures to which this animal was subjected.

The animal was killed when a tumor measuring 15 x 20 x 22 mm was discovered in the subcutaneous tissue of the right side of the chest. The tumor was uniformly pale gray, did not invade the overlying skin and did not metastasize. The animal was slightly obese. The thymus was atrophic. The spleen measured 22 x 7 mm in the 2 greatest lengths. The right adrenal was slightly enlarged and yellow. The other endocrine glands appeared normal save for the ovaries; the right was the site of a 4 mm sized tumor, predominantly a luteoma, and the left was atrophic. There was a minute adenoma in the lung.

Transplantation. The original tumor was transplanted into 11 male mice of the strain of origin. The tumors became palpable in all animals 4 months after transplantation and grew slowly but continuously. They measured approximately 4 cm in diameter at the time of death 7 to 10 months following transplantation. Before study of the sections, the tumor, because of its site of origin, was presumed to be a mammary tumor; consequently to test its hormonal dependency the testes of 5 tumor-bearing hosts were removed. This procedure in no way altered the rate of growth of the tumor. There was a conspicuous absence of metastases and lack of necrosis even though the tumor masses weighed as much as 8 to 10 g each. There was also a remarkable lack of secondary effect on the host. Even the thymus, indicator of stress, was only slightly or moderately atrophic in the animals with smaller tumors which were sacrificed for transplantation. Testes, seminal vesicles and endocrine organs, including the adrenals, appeared normal. There has been no change in the features of this tumor in the course of subsequent transplantations. Thus far there has been no failure to take and no tendency for the tumor to assume malignant features.

The second generation grafts were made in 2 passages on 14 female and 7 male mice all with success. Seven females received subcutaneous pellets of 7 to 8 mg diethylstilbesterol (containing diethylstilbesterol and cholesterol in the proportion 1 to 9). This treatment, given at a time when the tumor was thought to be of mammary origin, had no effect on the growth of the tumor. The first successive passages now in progress do not indicate any basic change in the features of the tumor.

All tumors were well circumscribed but invaded muscle tissue. Microscopically the only cells observed were mast cells. They contained varying numbers of granules of different sizes, which could be deeply stained with toluidine blue or Giemsa stain (Figs. 1,2). Granulation of the cells was as heavy as in normal tissue mast cells, from which they were indistinguishable. Microscopic examination of the draining lymph nodes disclosed no other change than the presence of a few normal mast cells (Fig. 3).

Assays. For determining the histamine, heparin and 5-hydroxytryptamine content of the tumors a weighed piece of tissue was minced finely with scissors and homogenized in water or isotonic sucrose solution with a glass tissue grinder until no fragments of tissue settled on standing for several minutes. *Histamine determination*: An equal volume of normal hydrochloric acid was mixed with a portion of the homogenate and the precipitated protein was centrifuged down. The supernatant was neutralized with normal sodium hydroxide and diluted with Locke solution. The extract was assayed for its histamine content using the isolated terminal ileum of the guinea pig suspended in Locke solution containing 6 mg of atropine per liter. *Heparin assay.* A solution of trypsin in phosphate buffer at pH 8 was added to a portion of the homogenate. This was incubated at 38° for 1½ to 2 hours. After incubation an equal volume of normal sodium hydroxide was added

to the solution. It was then neutralized and assayed for its heparin activity by determining at what dilution it would inhibit clotting in the *in vitro* system described below. Globulin was prepared by adding an equal volume of saturated ammonium sulphate to fresh, oxalated dog plasma. The precipitate obtained by centrifugation was dialysed for 24

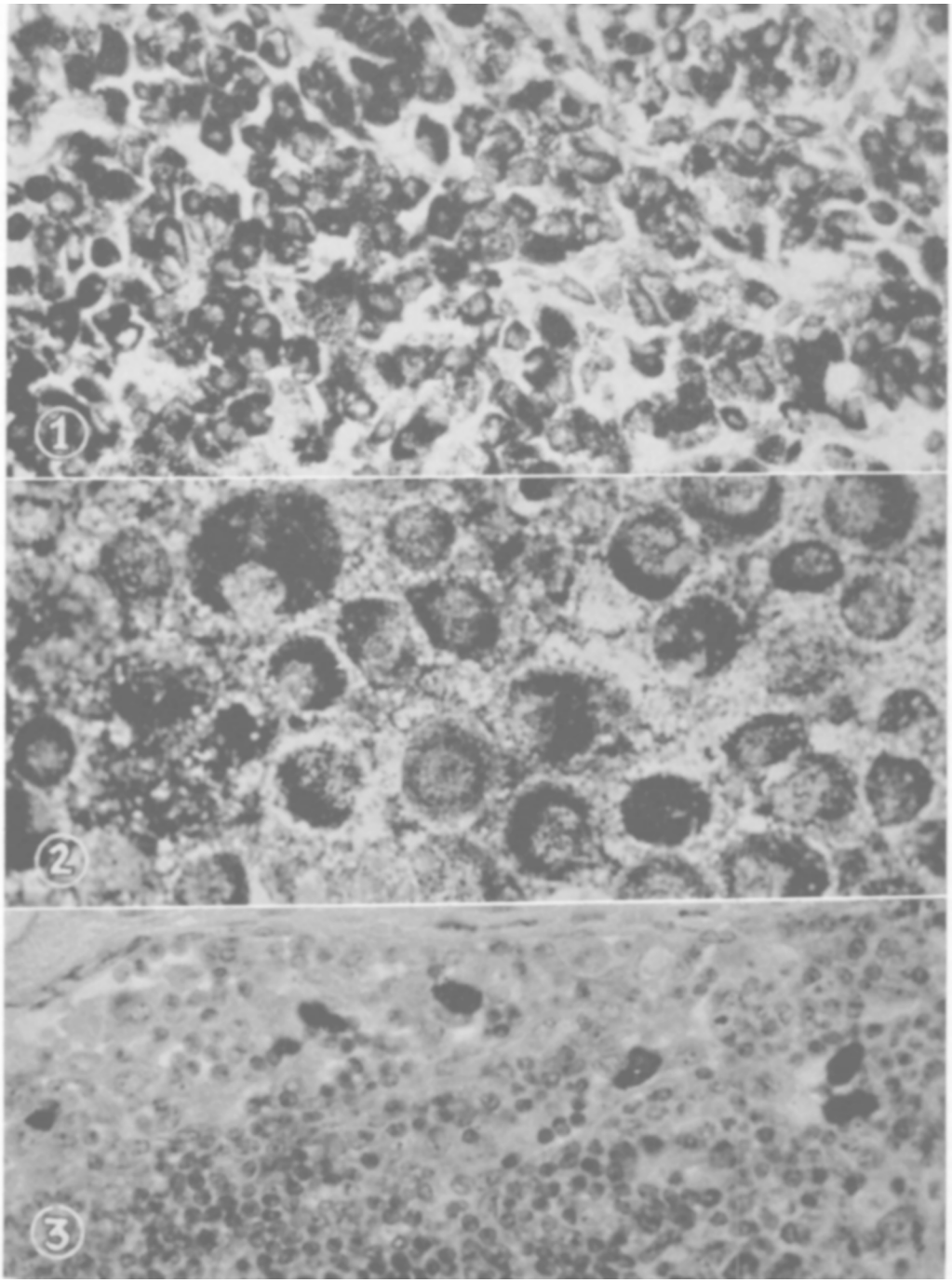

FIG. 1. Section of tumor cells stained with Giemsa stain. ×500.
FIG. 2. Imprint of mastocytoma stained with toluidine blue. ×1000.
FIG. 3. Section of a slightly enlarged lymph node in drainage region of a mastocytoma stained with Giemsa stain. ×370.

hours with frequent changes of distilled water. Thrombokinase solution was prepared as a watery extract of acetone dried powder of rat brain. By trial and error it was found that 0.3 ml of globulin solution + 0.3 ml of thrombokinase solution + 1.0 ml water + 0.05 ml of a 5% calcium chloride solution gave a firm clot within 3 minutes at 37°. The formation of this clot could be inhibited by 2 I.U. of heparin but not by one unit.

5-Hydroxytryptamine detection and estimation. Perchloric acid was added to a portion of the homogenate to give a perchloric acid concentration of 0.4 N. The protein was eliminated by centrifugation. Potassium hydroxide was added to the supernatant until the pH was 7.5. The potassium perchlorate was thrown down by centrifugation and the supernatant was applied to a column of Amberlite XE96 cation exchange resin in the sodium form. The column was washed several times with distilled water. The amines were eluted with 2 N hydrochloric acid. The eluate was neutralized with sodium hydroxide and added to excess acetone to make a solution of 95% acetone with 5% water. The insoluble inorganic salts were removed by filtration and the 95% acetone filtrate evaporated to dryness. The dry residue was dissolved in approximately 0.5 ml of water. This watery extract was applied at several spots 3 cm from the edge of a large sheet of Whatman No. 1 filter paper for paper chromatography. Standard histamine and 5-hydroxytryptamine solutions were also applied to the starting line 3 cm from the edge of the paper. The solvent for chromatography was butanol acetic acid and water. After 12 to 15 hours of ascending chromatography the paper was dried. A strip corresponding to point of application of one portion of the extract was cut out and the rest of the paper sprayed with a solution of 0.2% ninhydrin in acetone containing glacial acetic acid(9). After heating the paper for 2 minutes at 100° a very large blue spot due to histamine was seen close to the starting line. A brown spot was seen about half way up the paper. These spots corresponded in position to the histamine and 5-HT standards. In addition, the 5-HT spots showed the characteristic fluorescence of 5-HT when examined under UV light

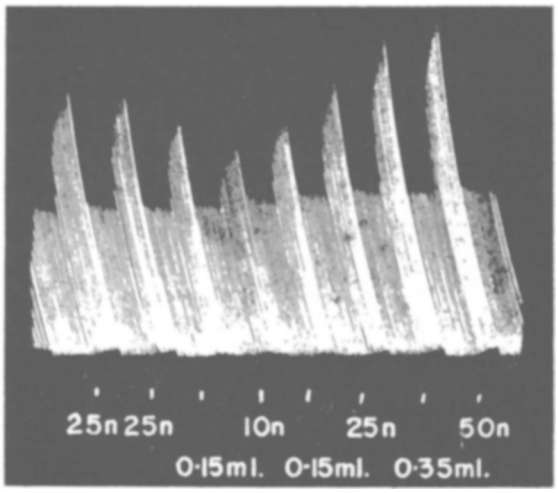

FIG. 4. Portion of 5-HT assay on isolated clam heart of material from 5-HT position of chromatogram from Exp. 6 extracted with 250 ml water. Upper line shows addition to the bath of 25, 10, and 50 mμg of 5-HT; lower line shows the addition of 0.15 and 0.35 ml of the sample.

(9). The piece of the non-sprayed paper corresponding to the 5-HT position on the sprayed paper was removed and extracted in distilled water. After appropriate dilution with sea water this solution was assayed for its 5-HT content on the isolated clam heart (Venus mercenaria) suspended in sea water (Fig. 4).

Results. The histamine, heparin and 5-hydroxytryptamine contents of several tumors are shown in Table I.

Discussion. The quantities of heparin and histamine present in these tumors are about twice those found by Cass *et al.* in spontaneous mastocytomas in dogs(10). According to Riley(11) 1.29 mg/g was the highest value ever recorded for tissue histamine. With one exception all values for our mastocytoma are higher than this. The presence of 5-hydroxy-

TABLE I. Histamine, Heparin and 5-HT Content of Mouse Mastocytomas.

Animal No.	Histamine, mg/g	Heparin, units/g	5-HT, μg/g
1	.85	790	8
2	—	1540	—
3	3.36	247	*
4	2.75	249	—
5	4.2	500	—
6	3.3	660	140

A dash indicates that no determination was made.

* Detected but not quantitated.

tryptamine in mast cells, although not in mastocytoma, has been previously reported (3,12). The value 140 μg/g in our mastocytoma is the highest recorded in mammalian tissues apart from carcinoid tumors, in which values up to twenty times greater have been found.

Summary. 1. A transplantable mastocytoma of the mouse is described. The tumor is benign and composed of cells indistinguishable from normal mast cells. 2. It contains greater quantities of histamine and heparin than have previously been found in normal or tumorous mast cells. It also contains large amounts of 5-hydroxytryptamine.

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Eggs of Floodwater Mosquitoes (*Diptera: culicidae*) VI. Effect of Metabolites on Latent Embryos in Uncapped Eggs.* (23376)

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Latent embryos in intact eggs of floodwater mosquitoes become activated after a series of events called *conditioning*(1). Such embryos hatch quickly and uniformly when subjected to a reduction in oxygen in the medium surrounding the eggs. Reduction in oxygen is effected in nature by metabolism of embryos when eggs are crowded into closely confined sites beneath layers of leaves, or by metabolic activity of microbes dispersed among the plant debris. It has been accomplished in the laboratory by crowding(2), by microbes(3) by reducing agents(1,3,4,5) and by replacing the dissolved oxygen in the medium with nitrogen or other inert gases(3,6).

Activation of conditioned embryos in intact eggs is prompt when oxygen in the medium is reduced to the proper level and at the proper rate. This fact suggests that latency is terminated by a sudden alteration in metabolic activity of the embryo. In preliminary experiments involving the effect of inhibitors of respiration on hatching of the eggs of aedine mosquitoes, the chorion proved to be impervious to solutes. Compounds which were quickly lethal to free larvae did not kill embryos in the intact eggs, even after exposure to these agents for several days. On the other hand, embryos in eggs made permeable by brief exposure to solutions of sodium hypochlorite were killed by the same inhibitors. Because of the barrier effect of the shell, latent embryos deprived of their shells or parts of shells were used in the experiments reported here.

Methods. All experiments cited herein were performed with embryos of *Aedes trivittatus* (Coq.) This species is well suited to experimentation because it (a) is easily conditioned, (b) is readily obtained in large num-

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