PREPARATION AND EFFECTS OF AN ANTI-MAST CELL SERUM*

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Although the tissue mast cell has been studied intensively ever since it was characterized by Ehrlich in 1879 (1), practically nothing is known about its function in the organism. Thinking that this knowledge might be gained from a study of animals made free of mast cells, we have attempted to prepare an antiserum in rabbits that would specifically destroy the tissue mast cells in rats. This paper describes the preparation of such an antiserum and its morphological and biochemical effects.

Experimental Methods

Albino rabbits (2 to 3 kg.) were immunized with rat mast-cell preparations. The latter were obtained (2) by differential centrifugation of washings of the peritoneal cavities of adult Sprague-Dawley rats. Three ml. of Tyrode's solution containing 3 to 5 $\times$ 10^6 mast cells were injected intravenously into rabbits on each of days 1, 2, 3, 5, 8, 10, and 12. Thereupon blood samples were taken daily from the rabbits and tested for anti-mast cell activity. (Of various immunization procedures attempted this one yielded the most potent anti-mast cell serum.)

Three tests for anti-mast cell activity were used. The first was an interfacial precipitin test in which a single dilution of antigen was layered on undiluted antiserum. The antigen was prepared by disrupting peritoneal mast cells in distilled water and adding NaCl to a concentration of 0.9 per cent; the concentration of mast cells was 4 to 16 $\times$ 10^5/ml. The second test consisted of microscopic observation of mast cells in the transilluminated mesentery (bathed in antiserum) of the intact, anesthetized rat, and the third of histological examination of fixed and stained whole mounts of mesentery, abdominal skin, ear skin, and scrotum of rats previously injected intraperitoneally with 10 ml. of antiserum. Similar tests were carried out with normal rabbit serum and with a rabbit antiserum against rat liver. To prepare the latter, saline homogenates of livers from Sprague-Dawley rats were injected into albino rabbits seven times during a period of 2 weeks. Peaks of anti-liver activity, as determined by interfacial precipitin tests were found on the 10th day after cessation of injections and were maintained for about 1 week. During this time the rabbits were bled and the serum was pooled for use in the various experiments.

Histological examinations as outlined above were also carried out in a series of animals injected intraperitoneally 1 day to 2 months previously with the several sera. Adjacent samples

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of the same tissues were also assayed for histamine and in some cases for 5-hydroxytryptamine (3).

RESULTS

The several tests indicated the presence of anti-mast cell activity in the rabbit serum as early as 3 to 5 days after the last injection of rat mast cells. Highest activity was seen at 10 to 12 days and was maintained for about 1 week, whereupon it gradually declined. On about the 24th day, however, a second peak of activity was noted.

Striking reactions occurred in all of the tests for anti-mast cell activity. Marked precipitin rings formed within a few minutes after antigen (disrupted mast cells) was layered over the mast cell antiserum. No precipitation was observed with antigen and normal rabbit serum or anti-liver serum. No rings were formed with any of the three sera when disrupted rat white blood cells were employed as the antigen. Within 10 to 20 minutes after exposure of the intact, living mesentery to mast cell antiserum, the normally immotile cytoplasmic granules of the mast cell began to oscillate rapidly. At first only a few granules were active but gradually more and more became involved until at the end of 10 to 15 minutes all were so engaged. Swelling of the cell accompanied the granular activity and many cells broke open, spilling their contents into the adjacent tissue. The cytoplasmic granules of both the intact and disrupted cells stained metachromatically when toluidine blue (1:250 in Tyrode's solution) was applied to the preparations. The staining revealed vacuolations in many of the intact cells. Normal rabbit serum was without effect on the mast cells of the mesentery. Anti-liver serum, however, was followed by a sudden rupture of many of the mast cells. Unlike the response to mast cell antiserum, this was not accompanied by oscillation of the cytoplasmic granules and gradual swelling of the cell. Except for the effects upon mast cells the mesenteries appeared normal after treatment with the several sera.

Microscopic examination of the tissues of rats injected with mast cell antiserum revealed marked destruction of mast cells. The details of the development and repair of this damage were evident in the experiment in which tissues were sampled at various times after intraperitoneal injection of a potent mast cell antiserum. At one day after injection all of the mast cells of the mesentery and up to 50 per cent of those of the abdominal skin, scrotum, and ear were found to be broken open. Disruption continued in these tissues during the next few days and in some cases as much as 100 per cent of the mast cells were destroyed. Some of the non-disrupted mast cells appeared quite normal, while others contained large vacuoles and conglomerations of cytoplasmic granules. During the 1st week after injection much metachromatic material was observed in fibroblasts, macrophages, and leukocytes. At the end of 7 to 10 days, however, phagocytosis of the debris of disrupted mast cells was complete, there being no

metachromatic material loose in the tissue or within phagocytes. At this time there were no mast cells in the mesentery and few, if any, normal or abnormal mast cells in the other tissues. At 2 to 3 weeks after injection of antiserum new, small, and sparsely granulated mast cells appeared in the various tissues. By 4 to 6 weeks the number of mast cells was greatly increased and their appearance was essentially normal, and by 8 weeks repopulation with normal cells appeared to be complete.

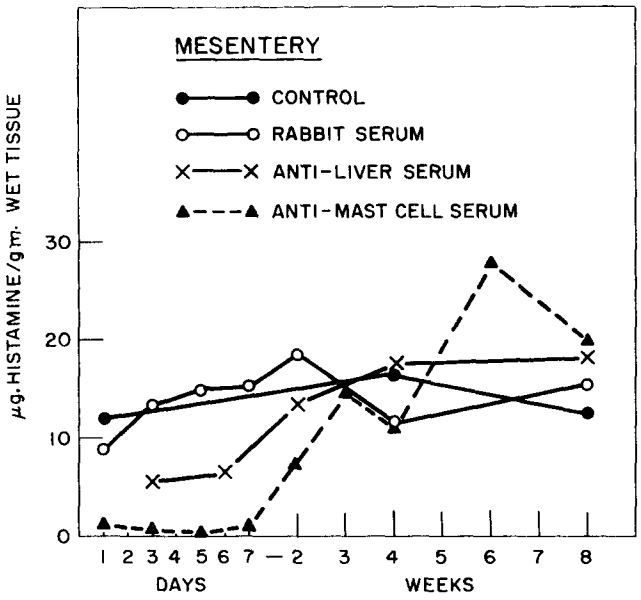

FIG. 1. Histamine content of mesentery following treatment with anti-mast cell, anti-liver and normal rabbit serum. Each value represents the average of estimates from 2 to 4 animals The same is true of the values in Figs. 2 and 3.

Normal rabbit serum was without apparent effect on any of the tissues studied. Injection of anti-liver serum, while evoking no changes in the abdominal skin, scrotum, and ear, was followed by disruption of all of the mast cells in the mesentery except those in the fatty regions.

Following the intraperitoneal injection of anti-mast cell serum, gross morphological changes were observed in the tissues of the peritoneal cavity. These consisted of shrinkage of the mesentery and shedding of parts of the capsules of the liver and spleen. The former changes were noted after about a week, whereas the latter were seen as early as 3 days after injection. At 2 weeks the mesenteries were further shrunken and shriveled, and fusion of the lobes of the liver and adherence of the spleen to the peritoneal wall and other adjacent tissues was apparent. By the end of a month widespread adherence of the viscera one to an-

other was pronounced; otherwise the individual organs appeared normal. At 2 months the mesenteries were less shrunken and more normal in appearance, but the visceral adhesions, though less pronounced, persisted.

Normal rabbit serum caused no gross morphological changes in the peritoneal cavity. Anti-liver serum, however, produced effects similar to but by no means as severe as those following anti-mast cell serum. Thus, the mesenteries were

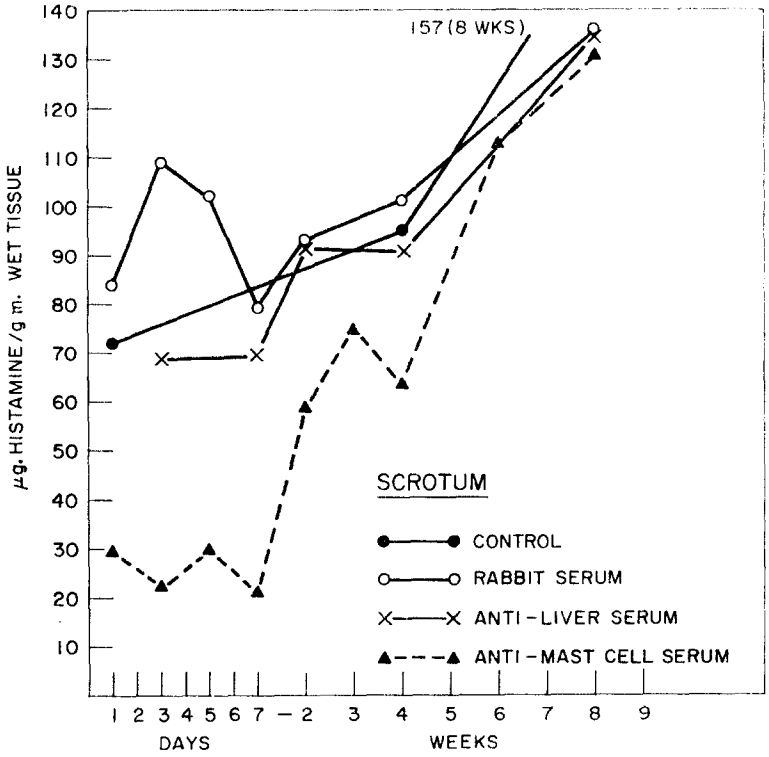

FIG. 2. Histamine content of scrotum following treatment with anti-mast cell, anti-liver and normal rabbit serum.

much less shrunken and the adhesions much less extensive and by the end of 4 weeks the organs of the peritoneal cavity looked practically normal.

It should be emphasized that in the above experiments only 3 ml. of anti-liver serum were injected intraperitoneally. This dosage was employed since it had been found that 10 ml. of anti-liver serum was followed by death of rats within 1 to 3 days.

Following the administration of anti-mast cell but not of anti-liver or normal rabbit serum, the skin of the feet, scrotum, muzzle, and ears took on a striking bright red color and became swollen. This was apparent as early as 1 to 2 hours after injection, most intense at 2 to 3 days, and absent 2 to 3 days later.

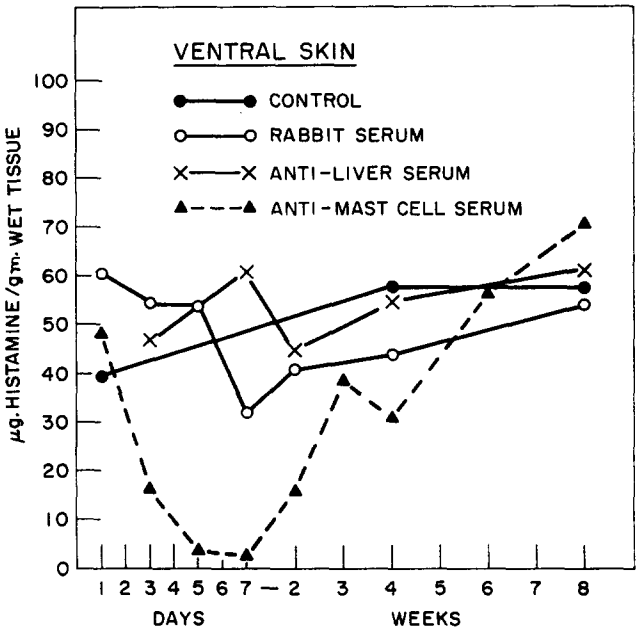

FIG. 3. Histamine content of ventral skin following treatment with anti-mast cell, anti-liver, and normal rabbit serum.

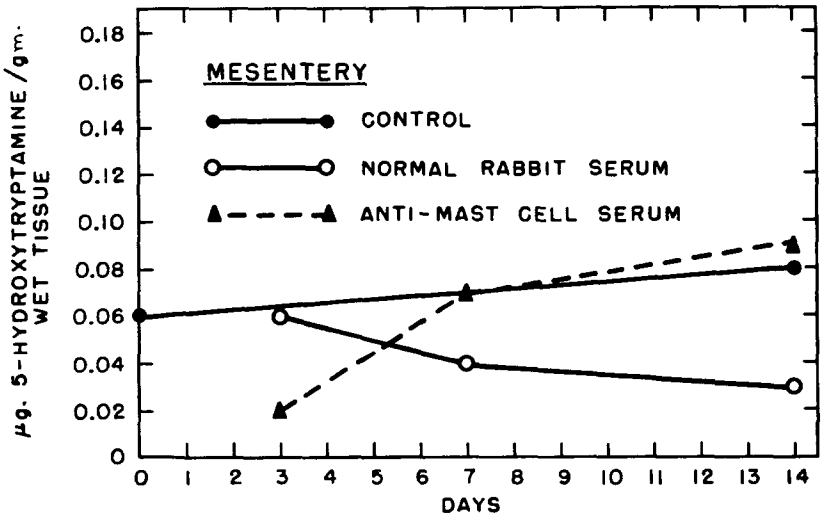

FIG. 4. 5-Hydroxytryptamine content of mesentery following treatment with anti-mast cell and normal rabbit serum. Each value represents the average of estimates from 4 to 6 animals. The same is true for Figs. 5 and 6.

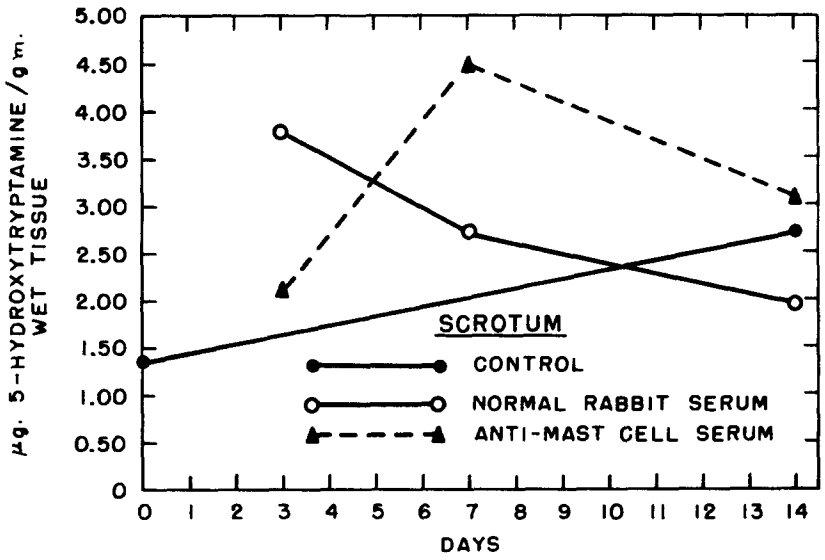

FIG. 5. 5-Hydroxytryptamine content of scrotum following treatment with anti-mast cell and normal rabbit serum.

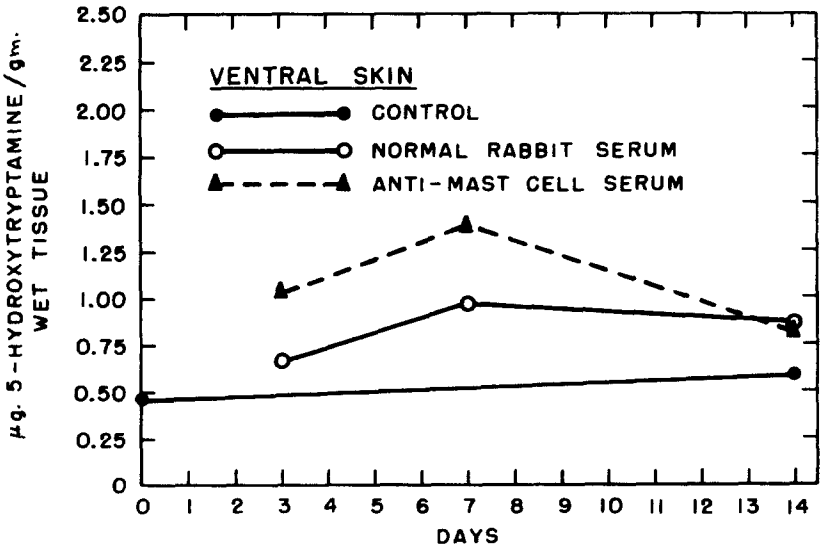

FIG. 6. 5-Hydroxytryptamine content of ventral skin following treatment with anti-mast cell and normal rabbit serum.

The influence of the several anti-sera upon the histamine and 5-hydroxytryptamine content of the mesentery, scrotum, and ventral skin is shown in Figs. 1 to 6. Histamine analyses were carried out in two additional experiments employing similar numbers of animals as those of Figs. 1 to 3, but sampling only on days 1, 3, 5, 7, and 14 after injection of anti-serum. The results confirmed the data shown in the present figures.

DISCUSSION

The present results show the practicality of preparing an antiserum that can efficiently destroy mast cells of the rat. This was indicated by the positive interfacial precipitin tests obtained against mast cell antigen with anti-mast cell serum but not with anti-liver or normal rabbit serum. Moreover, in the *in vivo* tests there was a distinctive response of the mast cells to anti-mast cell serum, and in the histological series destruction of mast cells in the scrotum, ear, and abdominal skin was evoked only by anti-mast cell serum.

The significance of the mast cell disruption following treatment with anti-liver serum in both the *in vivo* tests and the histological series is not immediately clear. It is possible that this effect may be due to an anti-mast cell component in the anti-liver serum; such could arise from mast cells contained in the liver tissue used as antigen for immunization. It is also possible that there are common antigens in both the rat liver- and rat mast-cell antigen preparations. Antibodies to such antigens could result in the destruction of mast cells found upon injection of anti-liver serum. It should be noted, however, that interfacial precipitin tests employing anti-liver serum and mast cells gave no indication that the anti-liver serum contains anti-mast cell precipitating antibody. Moreover, in tests on the mesentery *in vivo*, the distinctly different cytological changes following anti-liver and anti-mast cell serum suggest that the two sera act upon the mast cell by different mechanisms. It appears, therefore, that the basis of mast cell destruction by anti-liver serum might be something other than the presence of an anti-mast cell component in the anti-liver serum. We suggest that the effects of anti-liver serum on the mast cells may be secondary rather than primary, the anti-liver serum producing local changes or effects on other mesenteric cells that will result in the disruption of mast cells. Since the administration of normal rabbit serum was without effect in either the *in vivo* tests or the histological series, it would appear that the suggested local changes or effects on other mesenteric cells involve an antigen-antibody reaction.

In the histological series the failure of anti-liver serum to cause mast cell disruption in the fatty portions of the mesentery or in the scrotum, ear, and abdominal skin indicates that antibodies are not carried to those regions in amounts sufficient to produce either primary or secondary effects on mast cells. Anti-liver serum probably consists primarily of antibodies against rat antigens common to most rat tissues; it would be difficult for these to escape fixation by

the peritoneal tissues and the liver. Moreover, should anti-liver serum contain small amounts of specific anti-mast cell antibodies, one would expect that they would be bound readily by the accessible mast cells of the peritoneal cavity. (Intravenous injection of 2 ml. of the several antisera was without effect on the tissue mast cells.)

The gross morphological changes found in the peritoneal cavity following injection of anti-mast cell and anti-liver serum are not readily explained. It is possible that these changes depend upon the loss of mast cells from the tissues involved or that they are caused by reactions of the antisera with other cells of the peritoneal cavity. In favor of the former possibility is the indication of a return toward a generally normal appearance of the tissues as their repopulation with mast cells proceeds. Moreover, we have found in other experiments (unpublished) that the depletion of mast cells brought about in the mesentery of the rat by daily injections of the histamine liberator 48/80 for 1 month was accompanied by gross morphological changes in the tissues of the peritoneal cavity quite similar to those following anti-mast cell and anti-liver sera. On the other hand the general observation (4, 5) that destruction of the mast cells of the mesentery by the intraperitoneal injection of distilled water is not accompanied by other significant microscopic or macroscopic changes indicates that mast cell destruction *per se* is not sufficient to bring about the generalized tissue changes. Thus, it appears that in addition to their effects upon mast cells the antisera and 48/80 react with other tissue constituents and that these reactions are the bases of the general morphological damage.

The histamine analyses correlate well with the histological findings. Thus, decreases in the number of mast cells after treatment with anti-mast cell or anti-liver serum are accompanied by decreases in the levels of histamine in the same tissues; repopulation of the tissues with mast cells is attended by a return toward normal of the histamine content. These findings are in accord with the general observation (6) that the mast cells are the chief source of tissue histamine.

It is of interest to note that the histamine content of the scrotum of untreated rats underwent a considerable increase over the time course of the experiment. The significance of this change is not clear, since there appears (7) to be no general correlation between age and the level of tissue histamine. The rise in histamine was accompanied by a 50 per cent increase in the count of mast cells in the scrotum.

The measurements of 5-hydroxytryptamine were by no means as extensive as those of histamine. The results, however, clearly indicate that unlike histamine, the 5-hydroxytryptamine level does not vary directly with the mast cell content of tissues. This is analogous to the finding (8) that the intraperitoneal injection of polymyxin B is followed by destruction of mast cells and reduction of histamine but not of 5-hydroxytryptamine in the skin of the rat. The present results

confirm previous indications in the skin (3) that 5-hydroxytryptamine may be present in substantial amounts outside of the tissue mast cells. We have no explanation for the increase above normal levels of 5-hydroxytryptamine after treatment with anti-mast cell serum. Thus, at the time when the number of mast cells of anti-mast cell–treated rats was lowest the 5-hydroxytryptamine content had actually increased significantly above control values. These results indicate that under the present conditions 5-hydroxytryptamine may accumulate in the tissue outside of the mast cell or be present in abnormally high amounts in any mast cells that survive treatment with antiserum.

The marked reddening and the swelling of the skin of the ears, muzzle, scrotum, and feet following administration of anti-mast cell serum seems to depend upon the local disruption of mast cells, since only the injection of anti-mast cell serum was followed by destruction of mast cells in tissues remote from the site of injection (peritoneal cavity). Apparently histamine and 5-hydroxytryptamine released from the disrupted mast cells causes dilatation and increased permeability of the blood vessels in these regions.

The details of the phagocytosis of the debris of mast cells disrupted by antiserum are similar to those found in previous experiments (5, 9) in which mast cells were destroyed by distilled water, x-rays, ACTH, or cortisone. The beginning of repopulation of the tissues with mast cells occurs a few days later after treatment with immune serum than after treatment with distilled water (4, 5), but the general characteristics of the repopulation process are otherwise similar.

SUMMARY

In an attempt to obtain an antiserum that would bring about widespread destruction of mast cells in the rat, rabbits were immunized with mast cells isolated from the peritoneal cavities of rats. Striking evidence of anti-mast cell activity was indicated *in vivo* by mast cell disruption and *in vitro* by positive interfacial precipitin tests of the serum from rabbits so treated. The time course of the production of anti-mast cell activity in the rabbit serum was established. Normal rabbit serum was without effect on mast cells in the several tests. The same was true for rabbit anti-rat liver serum except when it was applied directly to tissues containing mast cells. In the latter case mast cell disruption ensued, but it appeared different from that evoked by anti-mast cell serum.

Intraperitoneal injection of anti-mast cell serum was followed by destruction of all of the mast cells of the mesentery and of the majority of mast cells in the ear, scrotum, and abdominal skin. The time course of this destruction, of the phagocytosis of the mast cell debris, and of the repopulation of the tissues with new mast cells was established. Attending the disruption of mast cells the levels of tissue histamine dropped sharply but returned toward normal as new mast cells appeared. In the scrotum and abdominal skin 5-hydroxytryptamine did not fall below normal and was markedly above normal at the time

when histamine and mast cell content were lowest. Reddening and swelling of the muzzle, ears, feet, and scrotum were present during the first few days after injection of the anti-mast cell serum. Intraperitoneal injection of normal rabbit serum was without any of the above effects. Similarly injected rabbit anti-rat liver serum had no effect on the mast cells or upon the levels of histamine and 5-hydroxytryptamine in the ear, scrotum, or abdominal skin. It caused no reddening and swelling of the muzzle, ears, feet, and scrotum. It was attended, however, by destruction of the mast cells and by a sharp fall in the histamine content of the mesentery. Gross changes in the tissue of the peritoneal cavity following anti-mast cell and anti-liver serum were described.

The possible significance of the several findings were discussed.

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