

ANAPHYLACTIC REACTION IN TUMOR-BEARING (MASTOCYTOMA) MICE

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THE MAST CELL of tissues of the experimental animal has been accepted as a source of 5-hydroxytryptamine (serotonin)¹ as well as being a reservoir for histamine, heparin, etc. The widespread degranulation of local tissue mast cells seen to follow injection of antigen into sensitized mice² suggests that these cells participate in the anaphylactic reaction by release of these mediators. However, an uncertainty concerning the causal relationship exists, for it has been noted that anaphylactic shock is little altered by previous depletion of histamine and serotonin from the tissues.³ The concurrent sensitization of the mouse to the lethal effects of histamine⁴ and anaphylaxis⁵ by inoculation with *Hemophilus pertussis* phase I organisms has led to speculation concerning the interdependence of these phenomena.⁶ The subsequent finding^{7, 8} that the pertussis-inoculated mouse possesses a concomitant sensitivity to serotonin has added the latter as another agent participating in anaphylaxis. Availability of a mouse-transferable mastocytoma, potentially capable of secreting large amounts of histamine and serotonin,⁹ provided the opportunity of observing the effect of pertussis-inoculation and subsequent challenge on the structure of the mast cell.

MATERIALS AND METHODS

Mice.—Adult, female mice of the LAF₁ strain, weighing about 15 to 18 grams, at the start of the experimental study, were each inoculated intraperitoneally with a saline suspension of homogenized mastocytoma.

Assay.—Starting about thirty days after inoculation with tumor, assays of urinary 5-hydroxyindoleacetic acid (5-HIAA) were carried out periodically on these animals. The pooled urine samples collected over a 24-hour period from groups of four to five mice in a fasting state were analyzed for 5-HIAA by the technique of Sjoerdsma and co-workers.¹⁰ This procedure was made semiquantitative by reading in the Beckman spectrophotometer at 540 m μ against a standard. This technique is reliable as a screening procedure since presence of

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an actively growing tumor is accompanied by liberation of relatively large amounts of serotonin and, hence, excretion of large amounts of 5-HIAA. The average amount of 5-HIAA excreted in 24 hours was calculated as gamma per mouse. About 50 per cent recovery of exogenously administered serotonin as urinary 5-HIAA is possible by this technique.

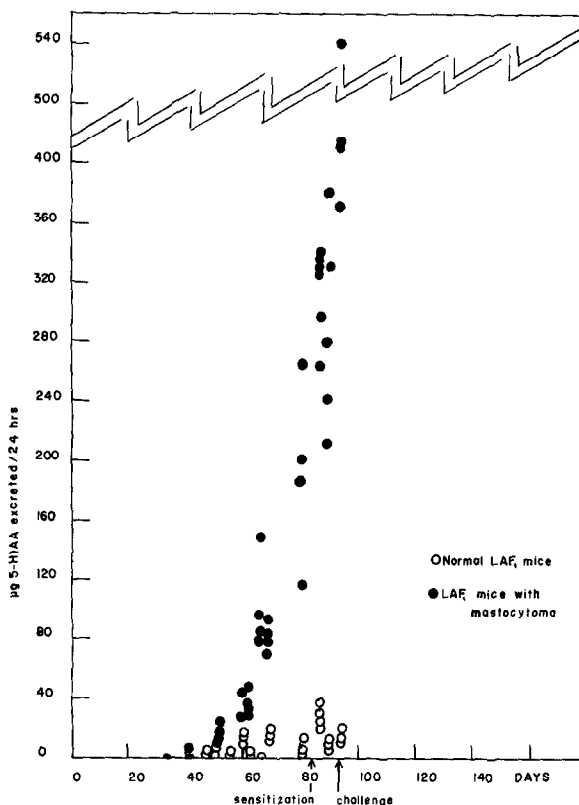


Fig. 1.—5-HIAA excretion in mastocytoma-LAF₁ mice.

Sensitization.—Each mouse was inoculated intraperitoneally with 6×10^9 *H. pertussis* phase I organisms on the eighty-fourth day following transplantation of tumor. By this time a marked increase in urinary 5-HIAA excretion had taken place (Fig. 1), usually coinciding with appearance of ascites and/or palpable tumor. Control mice received the vaccine only.

RESULTS

Histamine Sensitivity.—On the fourth day after inoculation with pertussis, each mouse of the following groups received 0.036 mg. histamine phosphate per gram of body weight intraperitoneally as a 0.2 per cent solution: (1) Swiss-Webster mice (control), (2) pertussis-inoculated Swiss Webster mice (control), (3) LAF₁ mice (control), (4) pertussis-inoculated LAF₁ mice, (5) tumor-bearing LAF₁ mice, and (6) pertussis-inoculated tumor-bearing LAF₁ mice. The results as seen in Table I indicate that neither pertussis-inoculated LAF₁

mice nor LAF₁ mice with actively growing tumor display any significant enhancement of sensitivity to histamine.

TABLE I. REACTION OF TUMOR-BEARING MICE TO HISTAMINE AND SEROTONIN

CHALLENGE (DAY 4 AFTER SENSITIZATION)	SENSITIZATION OF MICE					
	SWISS-WEBSTER		LAF ₁		LAF ₁ -TUMOR*	
	CONTROL	<i>H. pertussis</i>	CONTROL	<i>H. pertussis</i>	CONTROL	<i>H. pertussis</i>
Histamine Phosphate (mg./Gm. mouse)						
0.036	0/10†	5/10	0/10	0/6	0/12	0/6
.072				0/2		
.108				0/4		
.126				2/4	0/4	1/4
Serotonin (mg./Gm. mouse)						
0.05	0/4	5/5	0/5	5/5	0/4	

*Bearing palpable, actively secreting mastocytoma.

†No. dead/No. challenged.

Serotonin Sensitivity.—On the fourth day after the pertussis inoculation, each of the mice similarly prepared as in the first five groups described for histamine received 0.050 mg. serotonin per gram of body weight intraperitoneally as a 0.2 per cent solution. The results (Table I) indicate that pertussis inoculation sensitizes LAF₁ as well as Swiss-Webster mice to the lethal effects of exogenous serotonin. Pertussis inoculation failed to sensitize tumor-bearing mice to their own endogenously released serotonin. No higher incidence of deaths was noted over a thirteen day span following inoculation as compared to tumor-bearing mice not receiving the pertussis.

Anaphylaxis.—On the tenth day after inoculation with a mixture containing 6×10^9 *H. pertussis* organisms and 0.1 ml. horse serum given intraperitoneally each mouse of groups identical to the above was challenged by either 0.01 or 0.1 ml. horse serum given intravenously. The results, expressed as number of animals dead of anaphylactic shock/number of animals challenged (Table II), indicate that (a) the LAF₁ strain of mouse can be effectively sensitized by use of pertussis vaccine, (b) presence of an actively growing mastocytoma does not cause an enhancement of anaphylactic sensitization, and (c) the mastocytoma does not alter incidence of anaphylactic deaths as compared to either LAF₁ or Swiss-Webster controls.

TABLE II. ANAPHYLACTIC SENSITIVITY OF TUMOR-MICE

CHALLENGE (DAY 10 AFTER SENSITIZATION)	SENSITIZATION OF MICE					
	SWISS-WEBSTER		LAF ₁		LAF ₁ -TUMOR*	
	CONTROL H.S.†	<i>H. pertussis</i> - H.S.	CONTROL H.S.	<i>H. pertussis</i> - H.S.	CONTROL H.S.	<i>H. pertussis</i> - H.S.
Horse serum (intravenously)						
0.1 ml.	0/10†	9/10	1/5	3/5	0/5	4/5
0.01 ml.			0/10	3/7	1/12	9/15

*Bearing palpable, actively secreting mastocytoma.

†Horse serum.

‡No. dead/No. challenged.

Histologic Study.—Sections of tumor from animals of each of the following groups were fixed in formalin and stained with hematoxylin and eosin: (1) Control, receiving no treatment; (2) intraperitoneal horse serum, thirteen days prior to sacrifice; (3) intravenous horse serum challenge ten days after prior intraperitoneal horse serum, surviving shock; (4) same treatment as in previous group but dying in shock; (5) intraperitoneal pertussis and horse serum thirteen days prior to sacrifice; (6) intraperitoneal pertussis and horse serum, death by histamine on the fourth day; (7) intraperitoneal pertussis and horse serum ten days prior to intravenous challenge with horse serum, surviving, and (8) same treatment as those in previous group but dying in shock. In no case was the architecture of the tumor altered by the treatment. The tumor consists essentially of a sea of uniform appearing cells, generally small, with large vesicular nuclei and clumped chromatin. The cytoplasm is scant and moderately eosinophilic and contains a centrally located nucleus. With this stain, the cells resemble the lymphocytic-plasma cell series with no cytoplasmic granules. The tumor is moderately vascular and has a very thin, light, fibrous connective tissue stroma.

DISCUSSION

From previous studies¹¹ it can be stated that histamine actually plays a relatively minor role in anaphylaxis in the mouse. This conclusion seems warranted even though pertussis inoculation engenders an enhancement to both histamine and anaphylactic shock. The implication of serotonin as a mediator in the latter reaction, in part, arises from the simultaneous enhancement of its lethal effect in pertussis-treated mice.^{7, 8} In addition, the following observations stress its role: anti-serotonin agents inhibit the uterine reaction in sensitized mice,¹² mast cells disrupt and discharge their granules in response to an antibody-antigen reaction,² tissue serotonin is depleted following disruption of such cells,¹³ serotonin accumulates in the lungs of certain animals in anaphylactic shock,¹⁴ etc.

Although these observations seem to strengthen the role of the mast cell and serotonin in reactions of hypersensitivity, anaphylaxis apparently can take place fully as effectively in the pertussis-vaccinated mouse at a time when serotonin hypersensitivity has disappeared.¹⁵ Then, too, little alteration of shock was evident in rats depleted of skin and intestinal serotonin or treated with specific serotonin antagonists.³

Because of these varied observations, it became desirable to observe the anaphylactic reaction in mice under the influence of a tumor capable of secreting large amounts of serotonin. Inoculation with *H. pertussis* enhances the sensitivity of mice to exogenously administered serotonin, maximum sensitivity being attained about five days after treatment. Although the mastocytoma maintains a continuous secretion, pertussis inoculation was found to be without effect in engendering toxic reactions to this endogenously released serotonin over the course of the experiment. Possible explanations are (a) the amine as secreted in the native state is physiologically inactive by being in the bound

state, (b) inhibitory substances are present in the blood which equilibrate the activity, and (c) a resistance or tolerance is built up as the secretion progresses. The unavailability of the secretion to specialized sensitized sites should be considered, also, although serotonin is found in the blood.

The high concentration of 5-HIAA recovered in the urine, a direct reflection of the amount of serotonin secreted, indicates a high degree of activity of the tumor mast cells. If an enhancement of serotonin secretion resulted from the antibody-antigen reaction, the increment was not of sufficient magnitude to influence the 5-HIAA determination. No change in the integrity of the microscopic structure of the mastocytoma results from the anaphylactic reaction. Either the mast cells do not respond to the reaction, as has been observed elsewhere, or the reagents and/or the reaction products cannot penetrate the tumor to affect its cells.

As has been found previously with CF-1 mice,⁸ it has been here ascertained that the LAF₁ strain of mouse does not become highly sensitive to histamine after injection with pertussis. However, in both of the above strains the reaction to serotonin is enhanced and anaphylactic sensitivity is engendered by pertussis treatment. Although this may appear to indicate that serotonin is of direct significance in anaphylaxis, it has been shown with CF-1 mice that the latter reaction takes place in absence of serotonin sensitivity¹³ depending on the time interval following pertussis administration. The events occurring in this time interval bear investigation.

Parfentjev¹⁶ has reported that the presence of actively growing Sarcoma 180 sensitizes mice to histamine as does pertussis inoculation. No such effect was produced by the mastocytoma; it itself was without influence and its presence failed to augment the result of pertussis inoculation.

SUMMARY

Large amounts of 5-HIAA are found in urine of mice with an actively growing mastocytoma. This tumor, secreting serotonin in relatively large amounts, was no more lethal to pertussis-inoculated mice than it was to uninoculated control animals. Presence of the tumor was without effect on anaphylactic reaction or on sensitivity to histamine and serotonin. The LAF₁ strain of mouse becomes sensitive to serotonin but not to histamine following pertussis treatment.

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