Histamine in Intestinal Lymph of White Rat During Anaphylactic Shock.* (25899)

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Histamine has been shown by Mota(1) to appear in blood of sensitized white rats within a few minutes after intravenous administration of the specific antigen, horse serum. Strickland(2) later used egg albumin and *Bordetella pertussis* vaccine as sensitizing agents and also demonstrated an increase of histamine in blood of shocked rats. Sanyal and West(3) showed that severe shock can be produced consistently in the rat with *B. pertussis* vaccine together with egg albumin or horse serum. Subsequently, Sanyal and West(4) interpreted their findings as indicating that neither histamine nor 5-hydroxytryptamine participate in symptoms of anaphylactic shock in the rat. This conclusion was reached because shock ensued in animals even after skin and intestines presumably had been depleted of histamine and 5-hydroxytryptamine by preliminary treatment with polymyxin B sulfate, reserpine and total body irradiation. Neither mepyramine maleate nor BOL 148 seemed to reduce intensity of shock. Mota(1), however, had noted previously that antihistaminic drugs protected rats against anaphylactic shock. Parratt and West(5) suggested that release of histamine owing to polymyxin B sulfate is chiefly by injury to or destruction of mast cells in the peritoneal cavity and subcutaneous connective tissue. Histamine also is released from lungs. In their opinion polymyxin B sulfate releases maximal amounts of histamine and only small amounts of 5-hydroxytryptamine. Irradiation of the entire body destroys mast cells(6, 7). It is thought that irradiation depletes chiefly stomach and jejunum of histamine(4). However, Van Den Brenk(8) suggested that mast cells are resistant to radiation. Intraperitoneal injection of distilled water also destroys mast cells in the peritoneal cavity (9,10). Feldberg and Kellaway(11) and also Dale(12) pointed out that it is the quantity of histamine liberated at site of action which is important in production of pharmacologic activity. The small intestine seems to be the "shock organ" of the rat. This study was undertaken to determine whether histamine is released into the lymph that drains from gastrointestinal tract of rats during anaphylactic shock. Some preliminary studies are included regarding effect of polymyxin B sulfate, roentgen rays, and distilled water on release of histamine.

Method. White male rats of Sprague-Dawley strain weighing 150 to 350 g were used. Sensitization was carried out by simultaneous intraperitoneal administration of 25 mg of bovine gamma globulin (BGG) and 0.5 ml of *B. pertussis* vaccine. Twelve to 24 days later 10 to 12.5 mg of bovine gamma globulin was administered intravenously to induce shock. Shortly before inducing shock the mesenteric lymphatic vessel was cannulated with plastic catheter while animals were lightly anesthetized with ether, and a small catheter placed in tail vein. Lymph for use as a control was collected during immediate recovery period. The animal was then given an intravenous injection of 3 mg of pentobarbital sodium (nembutal)/100 g body weight. No. 60 plastic catheter was tied into the right external jugular vein of animals in which blood studies were done. A control blood specimen was secured from each animal and the shocking dose was then administered intravenously.

Lymph was collected for control period, usually 30 to 60 minutes, and for periods ending 6, 12, and 30 minutes after shock. Each specimen of lymph was heparinized and kept

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at 0° to 5°C until extraction, by the method of Code(13), or direct assay was carried out. Four rats were not given sodium pentobarbital during collection of lymph or during periods of shock. Histamine content of lymph of 12 rats was studied by extraction and assay. To secure specimens of at least 0.4 ml, the lymph from 2 rats was pooled. Histamine content of both lymph and blood plasma was studied in 11 rats by direct assay. Blood was collected during control period and at 6, 12, and 30 minutes after shock. It was drawn into a heparinized syringe and kept at 0° to 5°C until centrifuged at the same temperature. Plasma was separated and kept at 0° to 5°C until assay. Histamine content of lymph and blood was determined by assay using a segment of terminal ileum of the guinea pig suspended in 3 to 4 ml of atropinized modified Tyrode's solution(13). In specimens not extracted but directly assayed, each contraction considered due to histamine was blocked by mepyramine maleate. All results are expressed in terms of histamine base.

Six rats were studied after preparation with polymyxin B sulfate by the method of Parratt and West(5). The 3-day period of administration of the drug began on 10th day after sensitization. Animals were shocked on 13th day. Three rats were studied after irradiation of whole body 6 days after sensitizing dose by modification of the method of Eisen, Ellis and Wilson(6). The following factors were used in irradiation: 130 kv, 8 ma, 4½ mm aluminum filter, and distance 63 cm; 600 r was given to the back in 30 minutes and 400 r to central surface in 20 minutes. This gave a dose of 900 r to the center of body. Because only one of 12 rats so treated survived, the dose was reduced to a total of 700 r. Four rats were given this dose; 2 survived and were studied 13 days after sensitizing dose. Four rats were given 20 ml of triple distilled water intraperitoneally 6 days after sensitization with bovine gamma globulin and *B. pertussis* vaccine. One week later a study was made of lymph during shock. Because substances, other than histamine, which cause contraction of gut, were present in lymph and plasma, direct assay was only partially satisfactory and the results must be considered largely qualitative. The results of assays after extraction are more quantitative but not as exact as desired because of technical difficulties in making extracts with small amounts of lymph collected. Clinical shock was graded by dyspnea it produced and, in unanesthetized animals, by degree of restlessness as well. Gross appearance of the small intestine was used as chief pathologic criterion; the least degree of shock was indicated by slight congestion and the greatest degree by intense congestion.

Results. Bovine gamma globulin was a satisfactory antigen for sensitizing rats when given simultaneously intraperitoneally with *B. pertussis* vaccine. Animals displayed the most intense shock when shock was induced on 13th day after sensitization. The reaction of animals shocked after 21 to 24 days was much less intense.

Histamine was found in intestinal lymph in increased amounts after anaphylactic shock (Table I). This was demonstrated not only by direct assay but also by assays carried out after extraction for histamine. Histamine appeared promptly after shock, was greatly diminished after 12 minutes, and had returned to control values in 30 minutes. In general, the more intense the degree of shock, the greater the quantity of histamine found in intestinal lymph (Table I). During initial studies when direct assay was employed, animals which had little or no clinical or pathologic evidence of shock had little or no increase of histamine in plasma or intestinal lymph.

Rate of flow of lymph was carefully checked and increased 8 to 25 times during first 6 minutes after administration of shocking dose as compared to control period. The flow remained elevated or decreased slightly during second 6-minute period and returned to control rate in 30 minutes.

After shock, the quantity of histamine in lymph of animals pretreated with polymyxin B sulfate was definitely decreased (Table II). Degree of clinical shock in those animals seemed to have been modified. Flow of lymph, however, was nearly the same as in untreated animals.

TABLE I. Histamine Content of Intestinal Lymph of Rats after Anaphylactic Shock.*

Degree of shock†	Specimen, min.	Histamine,‡ μg/ml (expressed as base)
Poor	Control	.02
	6	.60
	12	.38
	30	.03
Fair	Control	.01
	6	.64
	12	.01
	30	.00
Excellent	Control	.02
	6	1.08
	12	.06
	30	.02
"	Control	.00
	6	1.12
	12	§
	30	§
"	Control	.00
	6	1.50
	12	.17
	30	.00
2 Fair	Control	.02
1 Good	6	.40
	12	.02
	30	.02

* Each histamine study represents lymph pooled from 2 animals.

† *Excellent*, considerable intestinal congestion, labored respiration; *good*, moderate intestinal congestion, labored respiration; *fair*, fair intestinal congestion; *poor*, little intestinal congestion.

‡ Extraction procedure used in all determinations.

§ Specimen inadequate for assay.

Pretreatment with polymyxin B sulfate alone caused death in 18 of the 32 rats so treated. All surviving rats lost 15 to 30 g.

Animals pretreated with roentgen rays became ill. Only one of 12 rats survived the dose of 900 r. Two of 4 rats given 700 r survived. No histamine was found in lymph of any of the 3 animals (Table II). They appeared too ill before shock to allow assessment of degree of clinical shock. The entire intestinal tract and peritoneum were hemorrhagic in each animal. Flow of lymph was reduced in 2 animals after shock but increased 10-fold in one rat.

Intraperitoneal injection of distilled water caused shivering in each of 6 animals so treated. In 4 animals shocked, the reaction was moderate but not extreme. Histamine content of lymph in one pair of animals may have been reduced somewhat below that noted in shocked animals without pretreatment (Table II). The increased flow of lymph during shock was the same as in untreated animals.

Comment. Sanyal and West(3) observed that shock was severest in rats sensitized with the aid of *B. pertussis* vaccine when the shock-inducing dose was given on 12th to 14th day rather than on 21st to 24th day. Our study confirmed their finding.

In general, intensity of shock after sensitization with bovine gamma globulin was not so severe as that produced by egg albumin, as reported by others(2,3). Sensitization with bovine gamma globulin, however, was suitable for our study as it permitted collection of lymph for 20 to 30 minutes after shock. The observation that there is a rough parallelism between degree of clinical shock and increased quantity of histamine suggests strongly that histamine plays an important role in production of anaphylactic shock in the rat. Undoubtedly other chemical mediators play a role.

Our efforts to deplete animals of histamine by preshock treatment with polymyxin B sulfate, roentgen rays, and distilled water can be considered as only preliminary. Although we followed the plan of administration of Sanyal and West(4) in giving polymyxin B sulfate, histamine was present in control lymph, and also was released after shock-inducing dose. The amount, however, was somewhat less than might have been expected with the degree of clinical shock observed.

Rats irradiated with roentgen rays were too ill to permit correlation of degree of clinical shock and quantity of histamine in lymph. In one rat we observed increase in flow of lymph usually seen in shocked animals, though no histamine was found.

Values for histamine in control lymph of all sensitized but otherwise untreated rats are similar to values in blood plasma. This was first noted in studies of blood and lymph done in the same animal by direct assay. Studies of lymph and plasma done by extraction method were done on different animals. Values of 0.015 to 0.026 μg of histamine/ml of

TABLE II. Histamine Content of Intestinal Lymph after Anaphylactic Shock Following Administration of Polymyxin B Sulfate, Roentgen Rays and Distilled Water.*

Pretreatment	Degree of shock	Specimen, min.	Histamine, μg/ml (expressed as base)
Polymyxin B	Good	Control	.03
		6	.24
		12	.05
		30	.05
"	Mild to none (shocked on 8th day)	Control	.03
		6	.00
		12	.12
		30	.01
"	Moderate	Control	.01
		6	.30
		12	.00
		30	.06
X-ray irradiation, 1 animal	None	Control†	.00
		6	"
		12	"
		30	"
" irradiation	"	Control	"
		6	"
		12	"
		30	"
Distilled water	Good	Control	"
		6	.80
		12	‡
		30	0
"	"	Control	.06
		6	.45
		12	.10
		30	.08

* Each histamine study represents lymph pooled from 2 animals.
† Histamine determined in this group of specimens by direct assay. Extraction procedure used in all others.
‡ Not enough to measure.

plasma are similar to values of 0 to 0.02 μg of histamine/ml in lymph.

Conclusions. 1) Bovine γ globulin is a satisfactory antigen for sensitizing rats when given intraperitoneally simultaneously with *Bordetella pertussis* vaccine. Intestinal lymph of animals so sensitized contains increased amount of histamine when collected 6 minutes after intravenous injection of shocking dose. The amount is roughly proportional to degree of shock. Rate of flow of lymph increases 8 to 25 times during the 12 minutes immediately after administration of shocking dose. 2) Lymph from mesenteric lymphatic system contains 0 to 0.02 μg histamine/ml, an amount similar to that in plasma. 3) A limited number of studies suggest that rats treated with polymyxin B sulfate and distilled water before shock were only partly depleted of histamine.

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Serum Folic Acid Activity by Improved *L. casei* Assay.*† (25900)

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Serum folic acid activity by microbiological assay with *Lactobacillus casei* has recently been proposed as a diagnostic test for folic acid deficiency(1,2,3). Levels less than 7.5 mμg/ml were reported indicative of the deficiency condition. Although folic acid activity of whole blood, plasma and serum had been extensively investigated by a variety of microbiological assays it had not previously been recommended as a means of assessing folic acid nutrition because activity levels in normals often were zero(4-14) and because the folic acid active compounds normally present in blood were presumably not folic acid or folinic acid(15). Nevertheless, because clinical folic acid deficiency is probably more common than generally appreciated and because laboratory means of assessing it are few(16), we undertook to evaluate the test proposed by Herbert *et al.*(1,3) and Baker *et al.*(2). In so doing, we found the assay they proposed lacked sensitivity at the lower levels which were of critical clinical importance. We wish to describe herein several improvements of the *L. casei* assay which greatly increase its sensitivity and accuracy and our experience with it in normal and folic acid deficient subjects. We found that levels in folic acid deficient subjects overlapped those of normals to such an extent that the assay had no clinical usefulness as a diagnostic test.

Materials and methods. Maintenance of stock culture. Lactobacillus casei ATCC 7469 was used in these studies, grown on the liver-tryptone agar of Nymon and Gortner(17) and maintained according to the scheme shown in Fig. 1. Three agar stabs, designated as monthly stabs, were prepared from the original culture. One monthly stab was used to prepare 4 weekly stabs, and the 2 remaining monthly stabs refrigerated until used. Each week 5 daily stabs were prepared from a weekly stab and the latter discarded. The remaining weekly stabs were refrigerated until used. A liquid broth inoculum was prepared each day from a daily stab, and the latter discarded. At end of month an unused monthly was employed to make 3 new monthly stabs and the scheme repeated. Spares were kept several months to be used in case of contamination in subcultures. *Inoculum preparation.* For liquid broth inoculum, 10 ml of skim milk medium previously described was used(18). A transfer from the daily agar stab was allowed to incubate at 37°C overnight (16-18 hours). 0.2 ml of uniformly suspended broth culture was transferred to a tube containing 6 ml of single strength assay medium to which 200 μμg folic acid had been added. This was allowed to incubate at 37°C for about 6 hours until O.D. of 0.1 to 0.12 was obtained in Coleman Jr. spectrophotometer at 660 mμ. The culture

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