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LIBERATION OF HISTAMINE AND SEROTONIN AND VASCULAR PERMEABILITY IN AN ACUTE ASEPTIC INFLAMMATORY FOCUS

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Degranulation of mast cells of a peritoneal suspension and of the mesentery of the small intestine and liberation of histamine and serotonin in albino rats with acute aseptic peritonitis were shown to begin during the first minute after injury and to reach a maximum at the fifth minute. By the 15th minute the concentrations of the free amines had fallen sharply and did not differ significantly from the initial levels. The dynamics of the immediate phase of increased vascular permeability corresponded to the dynamics of the free amines. The most marked increase in vascular permeability was observed at the 10th-15th minutes. By the 20th minute it was appreciably lower. Preliminary exhaustion of histamine and serotonin reserves reduced the degree of disturbance of vascular permeability only during the first 15 min after application of the inflammatory agent. It is concluded that histamine and serotonin cause disturbance of vascular permeability in acute aseptic peritonitis chiefly during the first 15 min after injury.

KEY WORDS: acute aseptic inflammation; mast cells; histamine; serotonin; vascular permeability

The principal mediators of the microcirculatory changes that characterize the initial phase of inflammation are histamine and serotonin. However, the duration of the period within which the action of these amines is the determining factor in the increased vascular permeability in a focus of acute inflammation has not yet been established.

Most of the data on the role of histamine and serotonin in the immediate phase of increased vascular permeability are based on their pharmacodynamic action. The dynamics of the mediators in the focus of inflammation directly after application of the inflammatory agent has not been studied.

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The object of this investigation was to determine the functional state of the mast cells of a peritoneal suspension and of the mesentery of the small intestine, the liberation of histamine and serotonin from them, and the changes in vascular permeability in the peritoneal cavity of albino rats in the course of acute aseptic peritonitis.

According to data in the literature [8, 10, 11], the mast cells are the sole source of detectable quantities of histamine and serotonin in the peritoneal cavity of albino rats.

EXPERIMENTAL METHOD

Experiments were carried out on 194 noninbred female albino rats weighing 150-220 g. Acute aseptic peritonitis was induced by intraperitoneal injection of a mixture of 0.05 ml turpentine and physiological saline (1 : 10). The animals were decapitated 1, 5, 15, and 30 min and 1, 2, and 5 h later, a peritoneal suspension was obtained [13], and it was immediately kept in the cold.* The mast cells were stained with neutral red and examined in a Fuchs–Rosenthal chamber under a magnification of the microscope of 400 ×. Their absolute and relative numbers and the percentage of degranulated mast cells were determined.

Mast cells in the mesentery of the small intestine were stained with toluidine blue [12] and also with safranine and alcian blue [14] and examined under a magnification of the microscope of 400 ×. The number of mast cells was counted in 100 fields of vision of the microscope and the percentage of degranulated cells calculated.

The histamine and serotonin concentrations were determined by modified fluorimetric methods of Shore and Snyder [1, 5] in the cellular and extracellular fractions of the peritoneal washings after removal of the turpentine and centrifugation of the washings at 350g and 4°C for 15 min [8]. The concentrations of the amines were expressed in micrograms per rat.

The state of permeability of the peritoneal vessels at various times after injection of the turpentine was judged from the concentration of intravenously injected (5 min before determination) trypan blue (0.75-1 ml of a 1% solution) in the peritoneal washings [2]. To obtain the washings, 5 ml of Tyrode solution was injected intraperitoneally. The concentration of dye in the washings was determined colorimetrically and expressed in g/ml washings.

To exhaust the reserves of histamine and serotonin bidistilled water was injected intraperitoneally in a volume of 10 ml/100 g body weight [9, 15].

EXPERIMENTAL RESULTS AND DISCUSSION

Marked degranulation of the mast cells of the peritoneal suspension and mesentery of the small intestine (81.14 ± 5.51 and 57.64 ± 8.67% respectively, compared with 1.20 ± 0.33 and 0.92 ± 0.26% in the control) was observed 1 min after injection of turpentine and was accompanied by liberation of histamine and serotonin, with an increase in their concentrations in the extracellular fraction and a decrease in the cellular fraction (Tables 1 and 2). The absolute and relative numbers of mast cells in the peritoneal suspension at this time did not differ significantly from the control ($992 \cdot 10^3 \pm 225 \cdot 10^3$ and 2.73 ± 0.49% in the control; $725 \cdot 10^3 \pm 164 \cdot 10^3$ and 2.34 ± 0.66% in the experiment).

No mast cells could be found in the peritoneal suspension 5 min after injection of turpentine as a result of their complete degranulation. The number of degranulated mast cells in the mesentery reached 65.90 ± 7.45% and the concentrations of free histamine and serotonin were maximal and exceeded the control level by 4.6 and 3.3 times respectively. However, by the 15th minute after injury the concentration of the free amines in the inflammatory focus no longer differed significantly from that in the control. At subsequent times of investigation (until the fifth hour) the concentration of the free amines showed no significant change. The level of intracellular amines continued to remain very low (Tables 1 and 2).

The permeability of the peritoneal vessels was sharply increased 5 min after injection of turpentine, when it was more than 4 times higher than in the control (Fig. 1). In the period between the fifth and 15th minutes it rose steadily and reached a maximum by the 10th to 15th minute, when it was 7 times higher than in the control. By the 20th minute a significant increase in vascular permeability was observed, although at this time and later during the investigation it continued to remain higher than in the control. For instance, 1-2 h after injection of the inflammatory agent the vascular permeability was 3 times higher than in the control. The

*The times of decapitation of the animals after injection of turpentine are given in the text and in the tables.

TABLE 1. Histamine Concentration (in μg per rat) in Peritoneal Washings of Albino Rats during Course of Acute Aseptic Peritonitis (M ± m)

Histamine	Control (n = 15)	Time after injection of turpentine						
		1 min (n = 12)	5 min (n = 12)	15 min (n = 14)	30 min (n = 8)	1 h (*n* = 13)	2 h (*n* = 12)	5 h (*n* = 11)
Total	7,91±1,53	5,61±0,93	4,18±0,97	1,78±0,34	0,85±0,24	1,39±0,37	0,98±0,33	0,60±0,23
P		>0,1	>0,05	<0,01	<0,01	<0,01	<0,002	<0,002
Free	0,82±0,23	3,25±0,59	3,76±0,97	1,47±0,27	0,49±0,15	0,51±0,10	0,27±0,07	0,21±0,08
P		<0,001	<0,001	>0,05	>0,1	>0,1	<0,05	<0,05
Intracellular	7,09±1,57	2,36±0,52	0,42±0,16	0,31±0,08	0,36±0,09	0,88±0,31	0,71±0,27	0,39±0,15
P		<0,05	<0,01	<0,002	<0,02	<0,01	<0,01	<0,01

TABLE 2. Serotonin Concentration (in μg per rat) in Peritoneal Washings of Albino Rats during Course of Acute Aseptic Peritonitis (M ± m)

Serotonin	Control (n = 7)	Time after injection of turpentine						
		1 min (n = 7)	5 min (n = 7)	15 min (n = 7)	30 min (n = 8)	1 h (*n* = 7)	2 h (*n* = 7)	5 h (*n* = 7)
Total	0,76±0,172	0,59±0,084	0,56±0,107	0,37±0,096	0,33±0,124	0,36±0,124	0,11±0,073	0,09±0,068
P		>0,1	>0,1	>0,05	>0,05	>0,05	<0,01	<0,01
Free	0,14±0,064	0,42±0,105	0,46±0,107	0,31±0,071	0,28±0,089	0,27±0,062	0,08±0,050	0,08±0,061
P		<0,05	<0,02	>0,01	>0,1	>0,1	>0,1	>0,1
Intracellular	0,62±0,169	0,17±0,048	0,10±0,023	0,06±0,036	0,05±0,036	0,09±0,066	0,03±0,023	0,01±0,009
P		<0,01	<0,01	<0,002	<0,001	<0,01	<0,002	<0,001

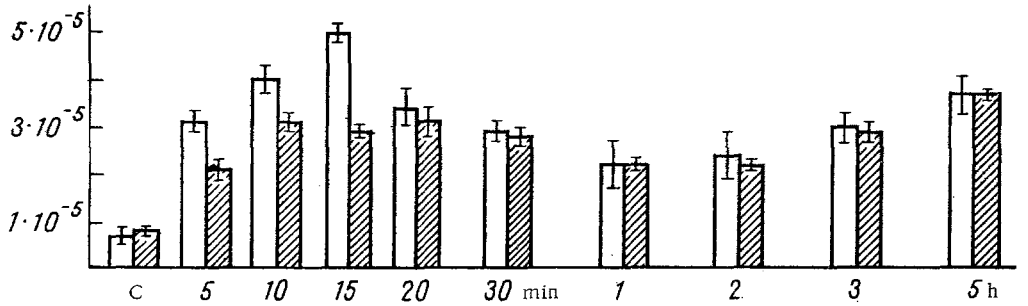

Fig. 1. Permeability of peritoneal vessels of albino rats in course of acute aseptic peritonitis without exhaustion (unshaded columns) and after exhaustion (shaded columns) of histamine and serotonin reserves. Abscissa, time after injection of turpentine; C) control; ordinate, concentration of trypan blue in peritoneal washings (in g/ml).

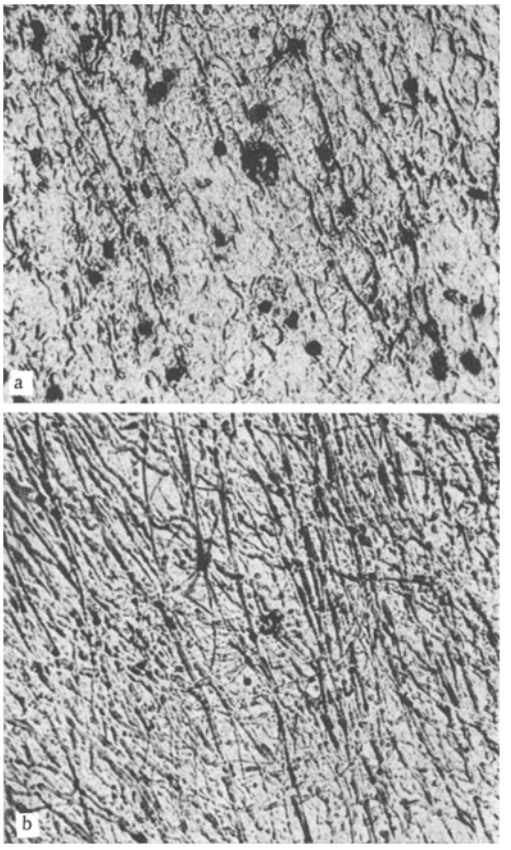

Fig. 2. Mesentery of albino rats under normal conditions (a) and 10 days after intraperitoneal injection of bidistilled water (b). Toluidine blue, 150 ×.

second, slow, phase of increased vascular permeability was found 5 h after injury. At this time it was significantly higher than that observed 1 and 2 h after the injection of turpentine and more than 5 times higher than in the control.

To confirm the role of biogenic amines in the increased vascular permeability in acute peritonitis, the permeability of the peritoneal vessels was studied in a separate series of experiments after preliminary exhaustion of the histamine and serotonin reserves. Intraperitoneal injection of bidistilled water, through its osmotic action, caused destruction and total disappearance of all the mast cells in the peritoneal cavity [9, 15].

The investigation was carried out 10 days after the injection of water. By this time the initial vascular permeability, having been disturbed by the injection of water, was completely restored (Fig. 1). No mast cells could be found in the peritoneal suspension or mesentery (Fig. 2). Very small quantities of free histamine (0.16 ± 0.018 μg per rat) were determined in the peritoneal suspension but serotonin was absent. The free histamine level was a little higher 5 and 15 min after injection of turpentine into the same animals (1.03 ± 0.207 and 1.11 ± 0.051 μg per rat respectively), but did not exceed its level in the intact rats ($P > 0.1$); no serotonin was found under these circumstances. These results are in agreement with data [9] showing that the substance 48/80, if injected intraperitoneally 7-10 days after an injection of distilled water, likewise does not liberate appreciable amounts of histamine.

Preliminary exhaustion of the intraperitoneal histamine and serotonin reserves reduced the degree of disturbance of vascular permeability only 15 min after injection of the inflammatory agent. Vascular permeability was reduced by more than 40% 10-15 min after the injection of turpentine compared with that found in animals without exhaustion of the reserves of biogenic amines. After 20 min and later the vascular permeability was the same in animals without and with exhaustion of the histamine and serotonin reserves (Fig. 1).

When the results of investigation of the concentration of biogenic amines and vascular permeability in the focus of acute inflammation are compared it will be seen that the most marked increase in vascular permeability is observed 5-10 min after maximal liberation of the amines. Electron-microscopic investigations [6] showed that histamine, injected intraperitoneally, causes opening of the endothelial spaces of the mesenteric microvessels and an outflow of colloidal ink from the vessels into the tissue for a period of 5-10 min.

The dynamics of the immediate phase of increased vascular permeability corresponds to the dynamics of free amines. The intensity of outflow of dye into the peritoneal cavity was appreciably reduced 15 min after injury. By this time the concentrations of free amines no longer differs significantly from their initial values. Preliminary exhaustion of the histamine and serotonin reserves reduced the severity of the disturbance of vascular permeability only for the first 15 min after injection of turpentine.

It can be concluded from the results of these experiments that histamine and serotonin cause increased vascular permeability in acute aseptic peritonitis mainly during the first 15 min after injury. The possibility of interaction between mediators in the focus of inflammation must be taken into consideration [3, 4, 7].

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