Role of Histamine in Endotoxin Shock.* (25843)

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Our recent work has been concerned with hemodynamic changes in animals following lethal injections of endotoxin(1-6). Similarity between vascular actions of histamine and endotoxin has been noted(4-7), suggesting that hemodynamic changes in endotoxin shock may be brought about by release of histamine. Our aim in this study was (a) to estimate changes in whole blood and plasma histamine following lethal injections of endotoxin, and (b) to determine if changes in vascular reactivity to histamine may result following endotoxin administration. Our findings suggest a prominent role of histamine in shock produced by endotoxin.

Methods. Histamine estimations were made with chemical procedure utilizing paper chromatography. Blood samples from catheterized femoral vessels of dogs, anesthetized with sodium pentobarbital (30 mg/kg), were collected directly into 4 volumes of acetone. Plasma samples were obtained from chilled centrifuged blood containing 1.7% potassium oxalate(8), plasma then added to acetone as above. Samples were refrigerated 12 hours, filtered until clear and evaporated to dryness, by warming and later by lyophilization. Dried residue was extracted with water and evaporated to standard volume < 1 ml. Equal aliquots of final solutions were placed on Whatman #1 paper and run for 10-16 hours in a solvent of n-butanol:acetic acid: water (4:1:5), using descending paper chromatography. Pauly reagent, followed by sodium carbonate overspray was used for color development(9). R_f's ranged between 0.10-0.14, and recoveries were 90-95% complete. Other procedures(10,11) were used for comparison with present technic and results were in close agreement. Changes in histamine concentration were estimated by comparisons of area X density of spots to pre-endotoxin values. Absolute concentrations of blood histamine were also estimated by comparisons to known standard concentrations, and all values before and after endotoxin ranged between 0.03 and 2.20 μg/ml. *E. coli* endotoxin (Difco), 2 mg/kg, was administered intravenously to anesthetized adult dogs. In second series, hindlimbs of dogs were perfused with heparinized blood continuously obtained from adult anesthetized dogs, as previously described(12). Intra-arterial injections of histamine (1 μg) were made in the isolated limb before and after intravenous injection of *E. coli* endotoxin (2 mg/kg, Difco) into the intact dog. Limbs were suspended on strain gauge weighing device and perfused at highest flow compatible with the isogravimetric state, flow remaining constant until termination of experiments.

Results. In Fig. 1 mean changes in plasma and whole blood histamine are shown following lethal injections of endotoxin. Within 2 minutes after injection there is a marked fall in whole blood histamine and a rise in plasma histamine. Plasma histamine remains

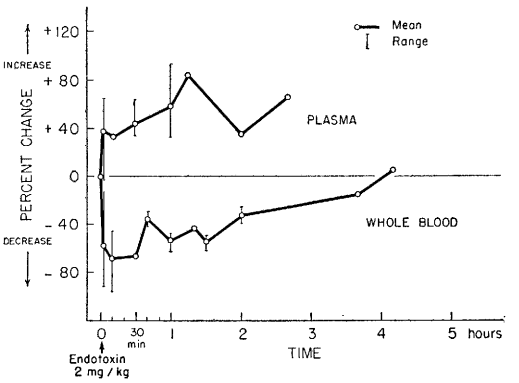

FIG. 1. % changes in histamine in 12 dogs following intrav. inj. of *E. coli* endotoxin (2 mg/kg). (6 dogs, plasma histamine, upper curve; 6 dogs, whole blood histamine, lower curve.) Mean values shown from zero time to termination of experiment.

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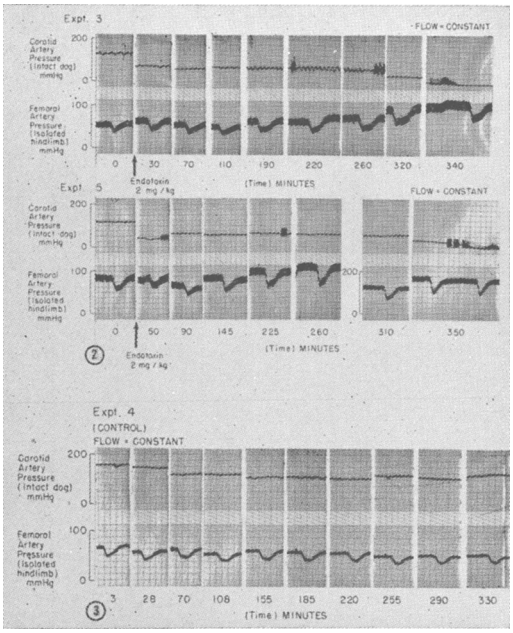

FIG. 2. Poly-viso recorded data from 2 separate whole dog-limb perfusion experiments (total 6 experiments). Responses of limb artery pressure to inj. of histamine are shown on lower panel of each section. Experiments terminated at death of animals.

FIG. 3. Control whole dog-limb perfusion experiment, as in Fig. 2, except that endotoxin is not administered.

increased during post-endotoxin period while whole blood histamine gradually returns toward pre-endotoxin level.

Recorded data from 2 separate limb perfusions are shown in Fig. 2. Vascular responses to injected histamine become progressively greater during post-endotoxin period, as contrasted with separate control experiment (Fig. 3) in which endotoxin is not administered and where no significant changes in histamine response are observed. In final 2 experiments (Fig. 4), comparisons are made of response to histamine when endotoxin and epinephrine (Suprarenin) are administered separately (flow constant). The rise in limb vascular resistance following injection of endotoxin (also seen in Fig. 2) is reproduced by constant infusion of epinephrine, 1-2 μg/min. A major difference exists between the 2 experiments, in that decrease in resistance to histamine becomes progressively greater after endotoxin, but less during infusion of epinephrine.

Discussion. Although there are limitations to the chemical procedure for histamine analysis(8,13,14), errors in the method do not invalidate measurements of *changes* in histamine(8) under special consideration in the present study. Changes in histamine concentration following lethal injections of endotoxin may be explained on the basis of release of histamine from the bound form in whole blood to the circulating form in plasma (15,16), or in addition, from histamine possibly released from tissue such as muscle(14). Our findings are consistent with results obtained from experiments concerned with anaphylaxis(16) and endotoxin(14), and further substantiate that the canine response to endotoxin may be anaphylactoid in type(17).

Progressive rise in limb vascular resistance after endotoxin may have occurred in response to the dual effect of increase in plasma histamine and increased responsiveness of certain vascular beds to circulating histamine. These additive effects could also explain the reported decreases in vascular resistance after endotoxin in various vascular beds(5,6). The implications of these findings support a hypothesis recently proposed by Schayer(18) regarding probable role of histamine in shock.

Summary. Intravenous injection of lethal doses of endotoxin in anesthetized dogs results in rapid decrease in total blood hista-

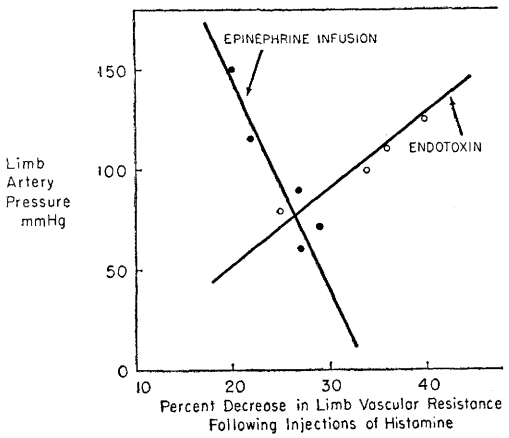

FIG. 4. Whole dog-limb perfusion experiments. Responses to histamine, as in Fig. 2, 3, following inj. of endotoxin and during infusion of epinephrine (2 separate experiments). Progressive rise in vascular resistance occurred in both instances. Decrease in resistance to histamine becomes progressively greater after endotoxin, but less during infusion of epinephrine, as vascular resistances are increasing.

mine, a concomitant rise in plasma histamine, and increased peripheral vascular responsiveness to histamine. These findings support the view that histamine performs a crucial role in progressive development of hypotension following administration of endotoxin.

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Circling Syndrome Produced in Mice by Dimethylaminohexose Reductone. (25844)

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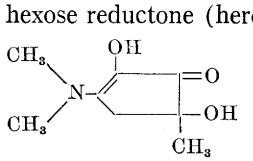
That certain amino-sugar reductones produce circling phenomena in rats, the movements suggesting those seen in waltzing mice, will shortly be reported by Ambrose, Robbins and DeEds of the U. S. Dept. of Agriculture who made the initial observations. After continued feeding the rats circled rapidly lasting many minutes, often set off by such stimuli as a bright light. Pulmonary hemorrhages and thyroid hyperplasia were observed but no primary central nervous or labyrinthine lesion was found. Lack of body fat, presumably from excessive exercise, was characteristic. In this paper reactions produced in mice by one of these reductones, dimethylaminohexose reductone (hereafter called reductone)



are reported.

Results. 1. *Induction of circling.* The circling syndrome was easily produced in white Webster mice by reductone. Table I shows effect of route and dosage on circling. Administration in drinking water or by injection (subcutaneous or intraperitoneal in distilled water or saline) was effective. Maximal effect was obtained after a single injection of 5 mg/20 g mouse, or 4 daily injections of 2.5 mg. At higher dosages early deaths occurred in some animals, and at lower dosages the syndrome was absent or incompletely developed.

2. *Circling syndrome.* Reductone symptoms were noticeable 3 to 4 hours after administration. The first, and most characteristic sign was head tossing, an upward jerk or shake repeated rapidly, accompanied by obvious difficulty in coordination and balance.