Influence of Histamine Liberator Substance 48/80 on Basophil Leucocytes of Rabbit Blood.* (23983)

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Different views about the functional relationship of basophil leucocytes and tissue mast cells have been published. Ehrich(3) and Asboe-Hansen and Kaalund-Jørgensen (1) expressed the view that blood and tissue mast cells may have some functional similarities. Mast cells(6) and basophil leucocytes (4) have been reported to contain histamine. A chemical histamine liberator of low toxicity, compound 48/80, which is a polymeric condensation product of p-methoxyphenethylmethylamine and formaldehyde, seems to bring about histamine release from tissue mast cells(7). This report concerns the effect of compound 48/80 on basophil leucocytes of rabbit blood.

Methods. Twenty-eight white male rabbits, of body weight between 2500 and 3000 g were used. The animals were kept in separate until they became accustomed to handling and blood sampling. Compound 48/80‡ in physiological saline was injected intravenously 1 mg/kg body weight. Injection was performed slowly in the marginal ear vein at about 9 a.m. Blood samples for basophil and total leucocyte counting were obtained from the ear veins just before injection as well as after 2, 4, 6, and 24 hours. Control counts were obtained from the same animals, getting corresponding volumes of physiological saline injected on a day within the previous week. A modified Moore and James diluting fluid(5) was used for direct basophil counting, consisting of: .05% toluidine blue in a phosphate buffer (pH 7.7) 40.0 ml; 96% ethanol, 5.5 ml; ammonium-sodium oxalate anticoagulant mixture, .5 ml. After mixing well, the fluid was filtered, and to it was added 0.5 ml of a saponin solution in 50% methanol (280 mg saponin in 10 ml alcohol). The fluid was filtered each time before use. Blood diluted 1:10 and Fuchs-Rosenthal counting chambers were used. Relative basophil counts (number of basophils/100 leucocytes) were obtained.

Results. All animals initially developed symptoms of distress such as prostration and difficult respiration, but with varying severity. Within half hour the animals appeared almost normal again.

The majority of treated animals demonstrated a fall in basophil counts compared to control counts (Fig. 1). Six hours after injection of compound 48/80 a decrease was observed in 23 animals; even after 24 hours 19 animals failed to regain their pretreatment levels.

The maximum drop in number of basophil leucocytes appears to take place 6 hours after injection of compound 48/80 (Statistical significance: $P<0.01$) (Fig. 1, Table I).

Discussion. Riley and West(6,7) reported that the histamine content of tissue mast cells may be released as a response to administration of different histamine liberators, including compound 48/80. They assumed a binding of histamine to the granular mucopolysaccharide content of mast cells, and thought that a granule release was the morphologic picture of the histamine release(7).

Asboe-Hansen and Wegelius(2) studied the effect of histamine liberators including com-

TABLE I. Change in Relative Basophil Counts (Avg for 28 Rabbits) following a Single Intrav. Inj. of Compound 48/80 (1 mg/kg).

Initial		6 hr after inj.	
Control	Exp.	Control (saline)	Exp. (48/80)
3.44	3.52	5.05	2.88

Statistical significance: $P < .01$

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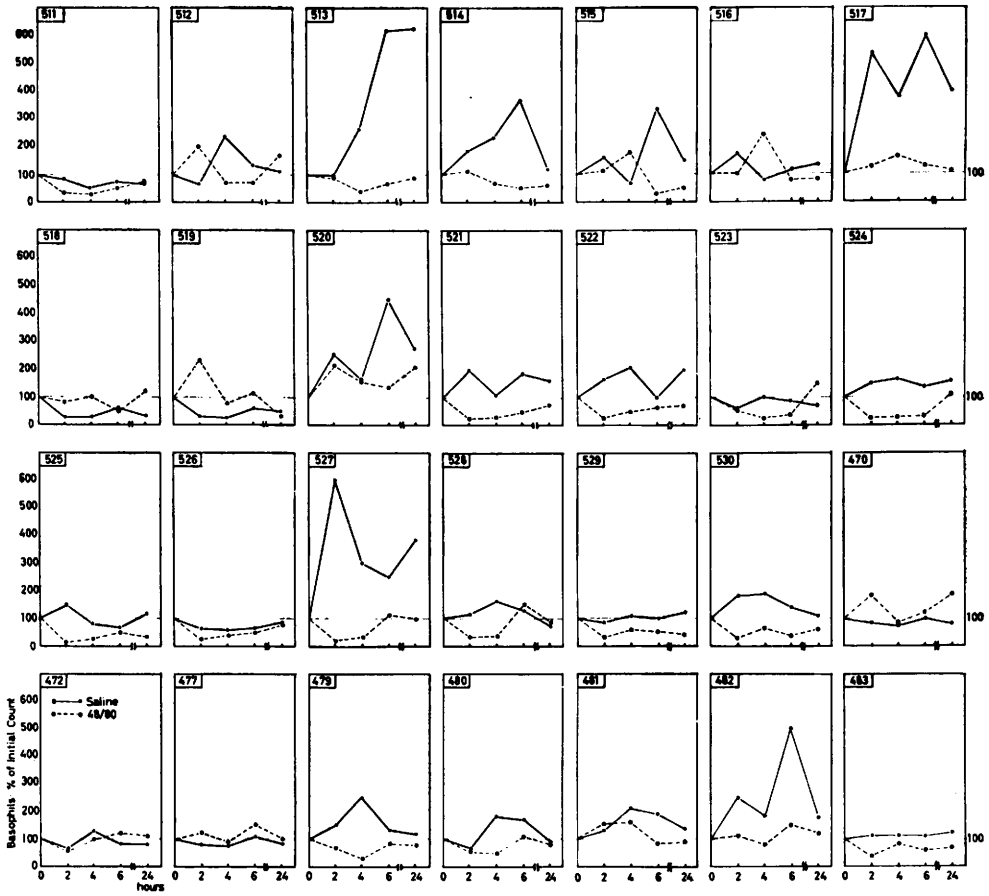

FIG. 1. Changes in relative counts of circulating basophil leucocytes in rabbits following a single intrav. inj. of 1 mg/kg of compound 48/80 (●——●). Control counts (●——●) were obtained, following inj. of a corresponding vol of saline in the same animal, on a previous day.

pound 48/80, on living connective tissue of the hamster cheek pouch. These authors reported a degranulation of living mast cells following systemic administration of compound 48/80. They interpreted the degranulation as a non-specific response to an increase in tissue water due to increased capillary permeability provoked by histamine liberated from some source. They suggested that the mucopolysaccharide originating from mast cells may bind water and thus convert the extracellular water to a ground-substance like hydrated gel.

If the histamine liberator injected has a direct action on basophils, these may release their metachromatic granules and thus escape detection; the basophil count consequently falls.

In the rabbit connective tissue mast cells are comparatively few, while the number of circulating basophils is relatively high. So, if a state of tissue oedema due to histamine liberation occurs as a response to administration of compound 48/80, and the few tissue mast cells are not capable of coping efficiently with such an emergency, the basophil leucocytes might migrate from the circulation to the oedematous tissues substituting their function of organizing the tissue oedema.

Summary. A decrease in number of circulating basophil leucocytes in rabbits occurred within 6 hours after injection of the histamine-liberator substance 48/80. The possible relationship between basophil leucocytes and connective tissue mast cells and especially a supplementary function of mucopolysacch-

aride release is discussed.

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Etiology of Chemically Induced Writhing in Mouse and Rat. (23984)

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Since it is generally agreed that analgesic compounds aside from opiates have little effect when tested by technics involving infliction of thermal or mechanical pain in normal experimental animals, many investigators have searched for methods which assess activity of "low potency" analgesics. Among these are Vander Wende and Margolin(9), and Siegmund *et al.*(7). The former group employed sodium iodomethamate intraperitoneally to produce "writhing" (a syndrome consisting of intermittent rubbing of the belly on the cage floor) in rats and arrived at oral PD_{50} values for opiates and antipyretic analgesics. This group blocked writhing by previous injection of procaine intraperitoneally. Siegmund *et al.* employed mice in a similar study using phenylquinone as inciting agent for writhing. 5-Hydroxytryptamine (5-HT) produces severe pain on intradermal injection (Armstrong *et al.*(1)) and has been reported (Lu, personal communication) to produce writhing when injected intraperitoneally in mice.

We studied effects of morphine, aminopyrine, lysergic acid diethylamide (LSD) and chlorpheniramine in prevention of writhing by phenylquinone, 5-HT and other substances, to arrive at the mechanism whereby writhing is produced and to ascertain optimal inciting agent of those mentioned above for efficient testing of new compounds.

Methods. Male Carworth mice weighing 18-22 g were distributed into groups of 5 animals each. Compounds under consideration as writhing producing agents were injected intraperitoneally, in aqueous solutions. Compounds investigated as blockers of writhing were administered subcutaneously, one-half hour prior to injection of the writhing agent. One group received only the writhing agent and served as control in each experiment. The usual period of observation for presence or absence of writhing was 10 minutes. If no response was observed the material was considered ineffective. If writhing was observed after this period, it was classified as "delayed." Such results are marked with an asterisk in the Table which gives number of animals which writhed, divided by total number in the group. Rats were also employed, weighing 150-200 g and treated in the same manner. Phenylquinone, histamine, which is commonly believed to play a role in inflammation, serotonin, heparin, and various releasers of these substances (Table I) such as L-1935, 48/80, and reserpine were used as writhing-inciters. NaOH and HCl were employed to produce pain, possibly without specific release of other substances. Distilled water and 0.9% NaCl solution were also tried. Writhing inciters were given in total volume of 0.25 ml/mouse or 1 ml/rat. Since our primary object was to attempt to elucidate the mechanism of action, we chose 4 drugs with different pharmacological activities, as possible blockers of the writhing effect: Morphine (a narcotic analgesic), aminopyrine (a non-narcotic analgesic), lysergic acid diethylamide (a blocker of serotonin), and chlorphe-