Release of Histamine-Like Substance from Mast Cells Following Intraperitoneal Administration of Fat Emulsion in Rats.* (25941)

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A disturbing though infrequent reaction to intravenous administration of triglyceride emulsions in patients is a syndrome characterized by skin flushing, dyspnea, sense of chest oppression, cyanosis and lumbar pain(1). These symptoms and signs are frequently associated with rapid rates of infusion and may begin within 1 to 5 minutes; they usually last 5 to 15 minutes and are followed by complete recovery. A subsequent infusion shortly after the reaction has abated or a few days later produces no further untoward reaction of this nature. The anaphylactoid nature of this syndrome suggests that the emulsions may cause release of histamine or other substances affecting smooth muscles. The present study was undertaken to clarify this hypothesis.

Materials and methods. Adult Sprague-Dawley rats were chosen for this study because it has been stated that the peritoneal tissue has abundent number of mast cells with high histamine content(2). Emulsions or solutions used were: (1) a 30% cottonseed oil emulsion‡ in water containing 5% glucose, 0.5% Demal 14, 0.25% Span 20, 0.5% Tween 60, 0.1% sodium cholate, 1% Ethofat C/15, 1% Aldo 28, and 1% purified soybean phosphatides; (2) Lipomul, I.V. (Lot No. 10680),§ a 15% cottonseed oil emulsion containing 1.2% purified phosphatides, 0.3% Pluronic F-68 and 4% glucose; (3) 5% glucose in water, (4) 0.85% NaCl; and (5) 48/80, a histamine liberator.‖ Each of the above materials was injected in 20 ml volumes intraperitoneally to animals weighing about 125 g and was left in the peritoneal cavity for 1-1¼ hours. The animals were then decapitated and fluids recovered from the abdominal cavity. The emulsions remained in a highly dispersed state and were examined for their effects on smooth muscles in the preparations described below. Samples of mesentery were fixed in methanol and stained with the Giemsa stain for microscopic examination of mast cells(4). *Preparation for demonstration of bronchiolar constriction.* Guinea pigs weighing about 500 g were pithed and respiration was maintained by air delivered under positive pressure through a tracheal cannula at 20 strokes per minute. A cannula introduced into the pleural cavity was connected to a tambour which recorded excursions of intrapleural pressures on a moving kymograph using the method described by Koessler and Lewis(5). Emulsions or fluids which had been recovered from the rat peritoneal cavity, as well as fresh materials, were injected into the left jugular vein of the guinea pig preparation. Bronchiolar constriction was indicated by sharp diminution in the excursions of the tambour lever. *Preparation for histamine bioassay.* The fluid recovered from the rat peritoneal cavity was assayed for histamine in a muscle bath containing a 2 cm segment of distal guinea ileum in 5 ml magnesium-free Tyrode's solution at 37°C(2). Atropine (10^{-6} M) was added to block spontaneous movements and cholinergic effects. Known amounts of histamine were added for quantitation of responses to the recovered emulsions. Amplitude of ileal contractions was recorded by kymographic tracings.

Results. Bronchiolar constriction. Fig. 1 shows the effect of fresh cottonseed oil emulsion and of emulsion recovered from the rat peritoneal cavity on the guinea pig preparation. The material from the rat peritoneal

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‡ Sources and chemical nature of emulsifying agents have been reported(3).

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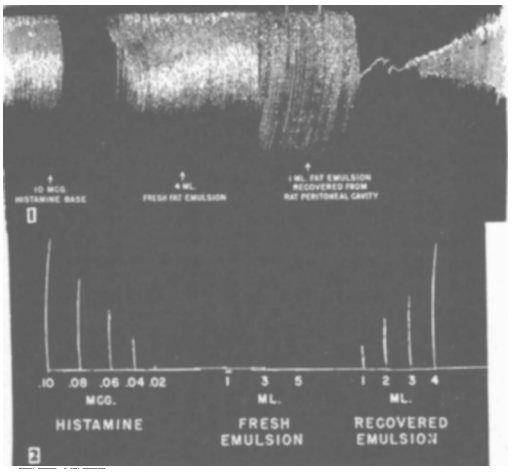

FIG. 1. Effect of fresh cottonseed oil emulsion, emulsion recovered from rat peritoneal cavity and histamine on bronchiolar constriction of guinea pig preparation.

FIG. 2. Effect of fresh cottonseed oil emulsion, emulsion recovered from rat peritoneal cavity and histamine on guinea pig ileal contraction *in vitro*.

cavity invariably caused extreme bronchiolar constriction characteristic of that observed following intravenous injection of histamine. Both the 30% cottonseed oil emulsion prepared in our laboratory and Lipomul, I.V., had this effect. Peritoneal fluid obtained by injection of 0.85% saline or 5% glucose and tested in amounts from 0.1 to 7 ml produced no such effect. When as much as 10 ml of fresh emulsion were infused only minimal respiratory changes were observed. Clustering of the particles of fresh emulsions by addition of bovine albumin also caused no bronchiolar constriction. In fact, whole cottonseed oil injected intravenously in amounts of 0.1 to 5 ml did not produce bronchiolar constriction, although the oil in large amounts was often fatal, presumably due to fat embolization.

Contraction of ileum. The assays for histamine in the peritoneal fluids using the ileal preparation are shown in Fig. 2. Four ml of the recovered emulsions produced contractions of the ileum corresponding to a content of 0.1 μg of histamine base. However, recovered fluids after intraperitoneal administration of 5% glucose or 0.85% saline contained no more than 0.02 μg/ml. The fresh emulsions caused no contraction of the ileal preparations. Addition of 0.1 mg of an antihistamine, Phenergan® (promethazine hydrochloride) to the muscle bath generally blocked the contractions caused by the recovered emulsions, although in occasional experiments this was not complete, suggesting presence of substances other than histamine causing smooth muscle contractions.

Changes in mast cells. Sections of mesentery showed greatly diminished numbers of mast cells after treatment with the emulsions and with 48/80. Many cells appeared to have been disrupted (Fig. 3). The peritoneal stroma did not appear otherwise altered. Normal mast cells were seen after injection of 5% glucose and 0.85% saline.

Discussion. Results indicate that intraperitoneal administration of triglyceride emulsions caused release of histamine, presumably from mast cells of the peritoneum. Since antihistamine did not always completely block activity of recovered emulsions, other vaso active substances such as 5-hydroxy-tryptamine may have also been liberated. These findings suggest that release of agents affecting smooth muscle may play an important role in development of "anaphyloctoid" reactions occasionally observed immediately following initiation of intravenous infusion of fat emulsions in patients. The results clearly indicate that the emulsion itself has no direct action upon ileal smooth muscle *in vitro* nor upon bronchiolar musculature in the pithed guinea pig.

It has been shown that non-ionic detergents of the "Tween" series release histamine when injected intravenously into dogs(6). The pres-

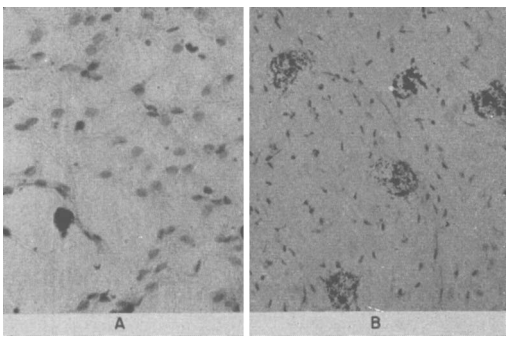

FIG. 3. A. Mesentery of a rat showing normal mast cells. B. Mesentery of a rat 60 min. after intraperitoneal administration of cottonseed oil emulsion showing disrupted mast cells (Giemsa stain).

ent study shows that cottonseed oil emulsion containing other non-ionic detergents are capable of releasing histamine and perhaps 5-hydroxy-tryptamine from tissue cells in rats.

Summary. Two triglyceride emulsions prepared for intravenous use in man have been examined for ability to release histamine from tissue mast cells in rat peritoneal cavity. The emulsions themselves had no direct action upon smooth muscle. Intraperitoneal injection of these materials caused rupture of the mast cells. The fluid recovered from the peritoneal cavity produced bronchiolar constriction in the pithed guinea pig and contraction of guinea pig ileum *in vitro,* indicating a significant histamine content. It is suggested that histamine release may play an important role in development of the occasional "anaphyloctoid" response following intravenous infusion of triglyceride emulsions in patients.

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Effects of Irradiation *in vitro* on Calcifying Mechanism of Epiphyseal Cartilage.* (25942)

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The calcifying mechanism of vertebrate bone, responsible for deposition of apatite crystals in the organic matrix, has been associated with a specific type of ground substance (1), mucopolysaccharide(2), a specific form of collagen(3,4,5) or collagen in combination with mucopolysaccharide(6). Proteins and polysaccharides have been found to undergo changes when irradiated with x-rays at sufficiently high dose levels(7,8). Thus, it was considered likely that irradiation could be used as a tool for study of the calcifying mechanism. The present investigation is a study of effects of irradiation *in vitro* on calcifiability and metachromatic staining properties of epiphyseal cartilage of rachitic rats. The metachromatic staining was undertaken on fresh sections to observe the system unaltered by histological technics. The effect of irradiation was also studied on rachitic specimens in which the initial phase of calcification had begun. Such sections will be referred to in this paper as nucleated.† Rachitic epiphyseal cartilage was used because it is a convenient system for study of the calcifying mechanism *in vitro* and has been used extensively for studying the intimate mechanism responsible for mineralization(9,10,11). When rachitic epiphyseal cartilage is incubated in serum of normal young animals, or in inorganic solutions containing physiological concentrations of calcium and phosphate, mineralization occurs only in the hypertrophic zone with the same histological distribution as occurs *in vivo.*

Materials and methods. Albino rats, 21 days old, were weaned and maintained for 3 weeks on a modified rachitogenic diet No. 2, U.S.P. The animals were then sacrificed by

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† Nucleated section refers to presence of nuclei of crystallization, which are initial submicroscopic aggregates of Ca and P which can serve as a center about which deposition of bone mineral may occur under appropriate conditions(13).