tein, pH, or other experimental conditions. It does appear from our studies, however, that SKF 525-A and DPEA not only inhibit enzymes of the mixed function oxidase system involving cytochrome P-450 but also those involved in the conjugation of fatty acids to 11-OH-Δ^9-THC and possibly other hydroxylated compounds, such as cholesterol, which require coenzyme A and ATP in the system. Since little is known about this liver microsomal esterase system, the knowledge that it can be inhibited by SKF 525-A and DPEA may be useful in future studies on the metabolism of certain drugs and other hydroxylated compounds.

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Pharmacology/Toxicology Section,
Battelle, Columbus Laboratories,
Columbus, OH 43201, *U.S.A.*

EDITH G. LEIGHTY

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Effects of compound 48/80 on dextran-induced paw edema and histamine content of inflammatory exudate

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It is well known that a local injection of dextran into the hind paw of the rat produces local edema. Pharmacological analysis using antagonists or depletors of histamine or of 5-hydroxytryptamine (5-HT) suggests that histamine and 5-HT are involved in the edema formation [1–3]. We reported recently that the highest concentration of histamine in the exudate collected from the swollen rat paw after injection of dextran occurred prior to the peak of edema, and that the concentration of the amine correlated with the severity of edema [4].

As to the fate of histamine released into the inflammatory site, the histamine is thought to disappear by diffusion into the circulation, as well as by enzymatic inactivation. Several studies suggest that putative mediators of inflammation diffuse into blood [5] or lymph [6–9] from the inflammatory site. Horakova and Beaven [5] have demonstrated clearly that appreciable amounts of released histamine diffuse into the circulation after thermal injury of the rat paw. Such a diffusion of released histamine may also occur during dextran-induced paw edema.

This short communication describes two subjects: (a) the effects of pretreatment of rats with the histamine liberator, compound 48/80, on edema formation and on the histamine content of the exudate and of paw cutaneous tissue after local injection of dextran; and (b) the determination of histamine content in blood plasma from the abdominal aorta and the saphenous vein after local injection of dextran.

Male Sprague–Dawley rats (Charles River Japan Inc., Atsugi, Japan), weighing 140–160 g, were used. Dextran was supplied by the Meito Sangyo Co., Nagoya, Japan. Histamine dihydrochloride was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Compound 48/80 was a product of the Sigma Chemical Co., St. Louis, MO.

Rats were injected intraperitoneally with compound 48/80, 1 mg/kg, four times during 24 hr; control animals received saline instead of compound 48/80. Dextran (average molecular weight 65,000) was dissolved in saline in a concentration of 4.0% (w/v), and 0.05 ml of this solution was injected into the subplantar region of one hind paw 2 hr after the last dose of compound 48/80. The paw volume was measured by a volume differential method before any injection and at various times after injection of dextran. Immediately after the animals were decapitated, incisions about 1 cm long were made on the dorsal and ventral skin of the paw. Without any squeezing, the exudate was then collected with capillary tubes (Propper Manufacturing Co., Inc., Long Island, NY). Pieces of cutaneous tissues, about 8 mm diameter, were obtained from the dorsal (two samples) and ventral (one sample) surfaces of the paw [5]. The tissue samples consisted of skin and the underlying soft tissue.

In another experiment, the concentration of histamine in plasma was determined after local injection of dextran or saline. Under ether anesthesia, blood was taken from the abdominal aorta and the saphenous vein using a needle and a plastic syringe containing 1/10 volume of 1.5% EDTA in saline. Plasma was obtained by centrifuging the blood at 4° at 2200 *g* for 20 min.

Histamine in exudate, paw tissue and plasma was determined by the extraction and fluorometric procedure of Shore *et al.* [10]. The concentrations of histamine in the exudate and plasma are expressed as μg/ml and ng/ml, respectively. The total amount of histamine in the exudate (μg) was calculated by multiplying the concentration of histamine in the exudate (μg/ml) by the increase in paw volume (ml). The histamine content of the paw cutaneous tissue is expressed as μg/g of wet tissue. All values of the amine in this paper are expressed in terms of the free base. The degree of paw edema is expressed as a percentage increase in paw volume relative to the initial volume.

The time course of paw edema formation and the his-

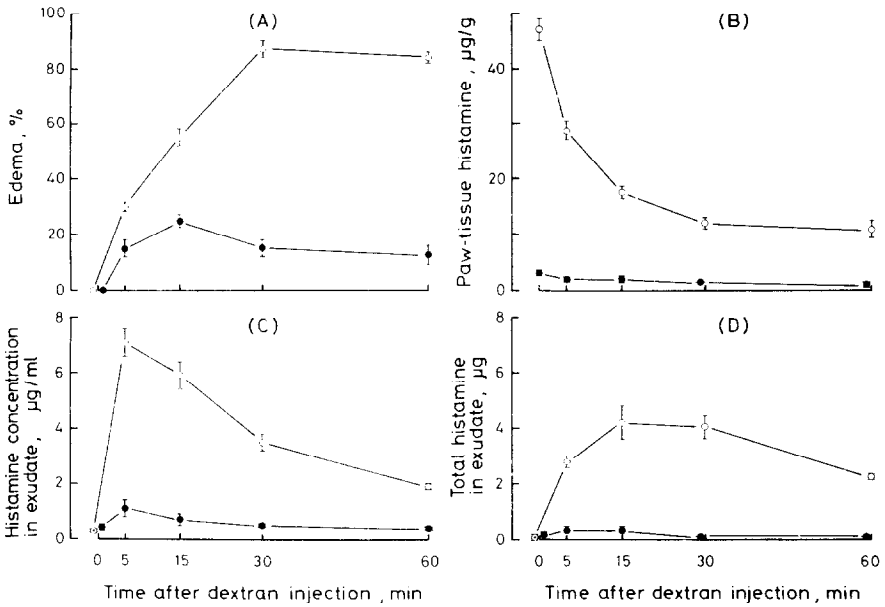

Fig. 1. Effects of compound 48/80 on paw edema (A), histamine content in paw tissue (B), and concentration (C) and total amount (D) of histamine in exudate after local injection of dextran. Rats received saline or compound 48/80 (1 mg/kg, i.p.) four times in the 24-hr period preceding local injection of dextran. Paw volumes of rats treated with saline (○) and of rats treated with compound 48/80 (●) before local injection of dextran were 1.34 ± 0.02 and 1.64 ± 0.02 ml (25.9 ± 1.6 per cent increase in paw volume compared with the value before the pretreatment with compound 48/80), respectively. The histamine value before injection of dextran (⊙) in control rats indicates histamine content in the fluid obtained by washing the subcutaneous space of paw with saline. Each point is the mean value of five to nine animals and the vertical bars indicate S.E.M.

tamine levels in the exudate and in paw tissue are summarized in Fig. 1. As shown in the previous report [4], the peak of edema was observed 30–60 min after injection of dextran in control rats. The peak of concentration (7.1 μg/ml) and the total amount (4.2 μg) of histamine in the exudate were obtained 5 min and 15 min after the injection, respectively. At these periods, the edema had not reached its peak. When the edema was at its peak, after 30–60 min, the concentration of histamine in the exudate had already declined. The total amount of histamine also had declined at 60 min after the injection. The development of edema was accompanied by a decrease in paw tissue histamine. Histamine content of paw tissue declined from 47 to 11 μg/g within 60 min after injection of dextran. Part of this decline was due to the increase in paw volume. When the values were corrected for this increase,* the decrease in histamine content was about 60 per cent. The greater part of the decrease in paw tissue histamine was observed within 15 min after injection of dextran.

Pretreatment with compound 48/80 alone produced some swelling of the paw (a 26 per cent increase compared with the value before the pretreatment) and marked reduction of paw tissue histamine. In rats pretreated with compound 48/80, dextran failed to produce the marked paw edema observed in control animals. In addition, the increase in histamine content of the exudate was slight. This indicates that the histamine appearing in the exudate after injection of dextran originates chiefly from mast cells in the paw tissue.

* Values were corrected as follows: (μg histamine/g tissue sample) × (paw volume after injection of dextran/paw volume before injection of dextran).

Compound 48/80, like other histamine liberators such as polymyxin B [11] and *n*-decylamine [11], releases not only histamine but also 5-HT from rat mast cells [2, 11–13]. 5-HT, which is an exceedingly potent substance for producing rat paw edema [1,4,14], has been suggested as a possible mediator of the paw edema produced by dextran [1–3]. The paw tissue of rats treated with compound 48/80 may have been depleted of 5-HT which might be released by local injection of dextran[2,13].

Although it has been proposed that kinins or prostaglandins play an important role in the development of paw edema produced by carrageenin [15,16] and thermal injury [17,18], the release of histamine in the early stage appears to be necessary to initiate the inflammatory reactions. The detailed studies of Horakova and Beaven [5] have provided evidence that histamine has a primary role in the early development of edema after thermal injury of the rat paw, and that its release has an important influence on the later stages of inflammation. The present experiments show that the major histamine release from paw tissue after local injection of dextran occurred in the initial stage before the peak of edema, and that when edema was fully developed there was little further release of histamine. In our earlier study [4], the amount of histamine appearing in the exudate correlated with the severity of edema. The present experiments also show that depletion of paw tissue histamine store by compound 48/80 reduced the appearance of histamine in the exudate and the formation of paw edema after local injection of dextran. Thus, our results suggest that the release of histamine is responsible for the paw edema formation by dextran. Histamine may play a role in initiating the edema formation.

The diffusion of released histamine into the circulation was also examined. An increase in histamine concentration

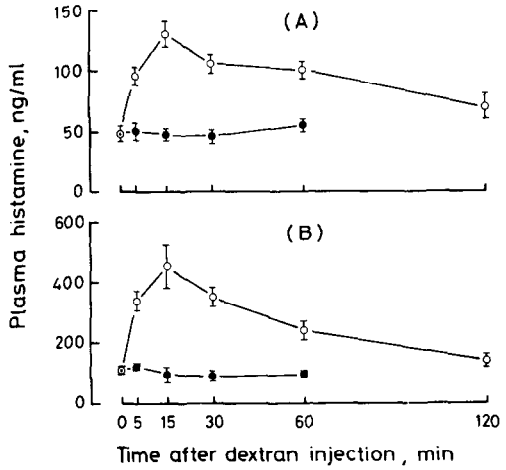

Fig. 2. Histamine concentration in plasma from rat abdominal aorta (A) and saphenous vein (B) after local injection of dextran (○) or saline (●) into the hind paw. The point (⊙) indicates histamine concentration in plasma from untreated rats. Each point is the mean value of five to twelve animals and the vertical bars indicate S.E.M.

was observed in plasma from the abdominal aorta and the saphenous vein after local injection of dextran (Fig. 2). The increase was greater in plasma from the saphenous vein than from the abdominal aorta. Therefore, the histamine appearing in plasma is thought to originate chiefly from the dextran-injected paw. The marked increase in histamine in plasma after injection of dextran indicated that appreciable amounts of the released histamine escaped metabolism and diffused into the circulation, being aided by the increase of local blood flow. Thus, in our experiment, the amount of histamine recovered from the inflamed paw would depend not only on the amount of histamine released into the inflammatory site but also on the rates of diffusion and metabolism of histamine, and blood flow. Since we do not know the contribution of these factors, it is impossible to determine in this study what proportion of the released histamine passed into the circulation and what proportion was recovered from the inflammatory site. However, the passage of the released histamine into the circulation may explain, in part, the disappearance of the released histamine from the inflammatory site.

Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, Tokyo 108, *Japan*

SHINICHI NISHIDA
SETSUO TOMIZAWA

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