of ACTH. The latter explanation is supported by the observation that this drug increases production of adrenal steroids in the intact animal both with normal, and with high levels of circulating adrenal steroids(6). The observations reported here might, on the other hand, be the result of an enhancement of the protein catabolic effects of the adrenal steroids by chlorpromazine. In support of a peripheral synergistic or additive action of chlorpromazine and cortisone, the findings of DeBias *et al.*, may be quoted; they found that doses of cortisone too small to protect completely a stressed, adrenalectomized rat, are much more effective when combined with subeffective doses of chlorpromazine(9).

Summary. Chlorpromazine at a dose level of 3.4 mg daily causes an increase of urinary nitrogen excretion and a decrease of weight gain in the intact tube fed rat. These changes are statistically significant. The protein catabolic effect of 5 mg of cortisone acetate administered daily is increased significantly by simultaneous treatment with chlorpromazine.

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Further Studies on Release of Serotonin and Histamine During Anaphylaxis in the Rabbit. (23258)

T. PHILLIP WAALKES, HERBERT WEISSBACH, JOHN BOZICEVICH AND SIDNEY UDENFRIEND (Introduced by R. W. Berliner)

Natl. Heart Inst. and Natl. Inst. of Allergy and Infectious Diseases, U. S. Dept. of Health, Bethesda, Md.

During anaphylaxis in the rabbit, there is a transient increase in plasma concentrations of both serotonin (5-hydroxytryptamine) and histamine(1). The increase occurs immediately after intravenous injection of the antigen, and indicates release of these amines from a bound to a free form within the animal. When an antigen is added to the whole blood of a rabbit previously sensitized to this antigen, concentrations of serotonin and histamine also increase in the plasma(1). In rabbit blood both serotonin and histamine are found almost exclusively in the platelets (2). Therefore, the rise in plasma concentrations of these amines, during the *in vitro* antigen-antibody reaction, must be due to their release from platelets. However, serotonin and histamine are found in a variety of rabbit tissues in addition to the blood platelets. The major portion of the body serotonin is found in the gastrointestinal tract; a much smaller amount is found in the lung(3) and the brain(4). Consequently, during *in vivo* anaphylaxis, the increase of serotonin and histamine in the plasma could be due to their release either from the platelets or tissues, or from both sites. The *in vitro* study, with whole blood, suggests that at least some of the free serotonin and histamine is derived from platelets. *In vitro* studies have shown that histamine is released when organs of sensitized rabbits are perfused with a solution of the specific antigen(5). Thus, it appears that in the rabbit histamine may be released not only from platelets but also from tissues, during anaphylaxis. Similar *in vitro* studies

have not been reported for serotonin. Whether in the living animal, during anaphylaxis, serotonin is released only from platelets, or from both platelets and tissues is not known. The present study is designed to help answer this question by using reserpine in sensitized rabbits to deplete their platelets of serotonin and histamine prior to the anaphylactic reaction.

Materials and methods. Rabbits were sensitized to horse serum by intraperitoneal injection of one ml of serum every day for 6 successive days. They were used in this study when their antibody complement fixation titers were high. The rabbits that were treated with reserpine were given the drug intravenously 19 hours prior to injection of the antigen. All the animals were anesthetized with nembutal and ether. Blood was obtained by means of a catheter inserted into a carotid artery, and collected in siliconized tubes. Heparin was used to prevent clotting. Plasma serotonin and histamine, and whole blood serotonin and histamine were determined as described previously(1). Serotonin and histamine were analyzed in tissues, which had been homogenized with 0.1 N HCl, by the same procedure used for whole blood. Standards, recoveries, and blanks were run with each series of determinations. Recovery of added serotonin and histamine from plasma is essentially quantitative with these methods; from whole blood and tissues recoveries are about 80 to 95%. Details of the analytical methods are being published in a separate communication.

Results. Reserpine, in a single intravenous injection of 5 mg/kg, causes a release of most of the serotonin from rabbit platelets and tissues within 19 hours(6). Histamine is also liberated from platelets(7) by reserpine but not in a significant quantity from rabbit tissues. In this investigation, it has been found that by reducing the amount of reserpine to 0.1 mg/kg, the platelets are depleted of serotonin and histamine to essentially the same level as by the larger amount of the drug. However, in contrast, the total concentration of serotonin in the tissues is not lowered appreciably by the smaller amount of reserpine. Thus, by injecting 0.1 mg/kg of reserpine intravenously into rabbits, sensitized to horse serum, their platelets could be depleted of serotonin and histamine without reducing the tissue content of these amines to any great extent. Therefore, any significant increase in the plasma concentration of serotonin and histamine during anaphylaxis in such an animal should be due to a release of these amines from tissues.

TABLE I. Amount of Serotonin and Histamine in Rabbit Whole Blood and Intestines 19 Hr after Reserpine as Compared to Control Values from Untreated Animals. Experimental values represent range or avg of at least 4 animals. (Range was used where the analyses differed by more than 1 γ/ml or g.)

	Serotonin (γ/ml or g)		Histamine (γ/ml or g)	
	Whole blood	Intestine	Whole blood	Intestine
Normal	3–5	6.5–16	2–4	4 –7
Reserpine, 5 mg/kg	.5	3*	.8	4.5–6
Reserpine, .1 mg/kg	.7	6.5–12	.7	

Pletscher, A. *et al., Science,* 1955, v122, 374.

The intestines in addition to the platelets are the principal source of serotonin in the rabbit(6). The amount of serotonin in the intestines far exceeds that found in any other organ, both as to total amount and per gram of tissue. Therefore, if any significant amount of the free serotonin in the plasma during anaphylaxis is derived from a tissue site, that site would be, most likely, the intestinal tract. As shown in Table I, the quantity of serotonin per gram of small intestine is high. However, even if a substantial release of serotonin occurs from the intestinal tract during anaphylaxis, it would be difficult to detect because of the wide normal variation (6.5-16 γ/g). Actual analyses of the intestines from 4 rabbits after anaphylaxis have shown that the amount of both serotonin and histamine is within the normal range. Previous work by Rose(8) revealed that the amounts of histamine in a variety of rabbit tissues following acute anaphylactic shock were not appreciably different from the amounts found in the same tissues of unsensitized control animals.

Table I gives the values for concentration

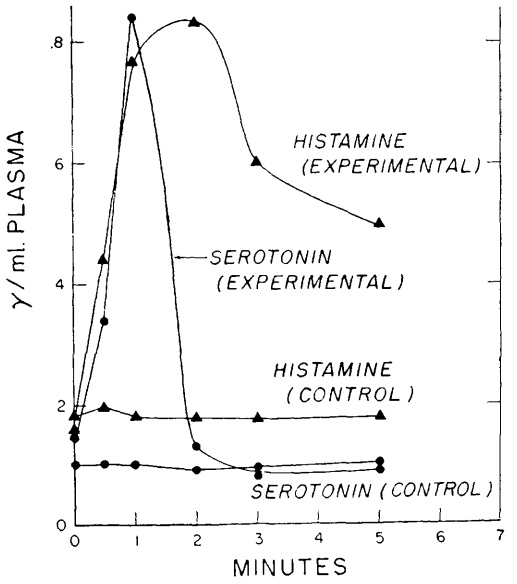

FIG. 1. Plasma serotonin and histamine in an unsensitized control rabbit and a sensitized rabbit following intrav. inj. of horse serum (2 ml/kg).

of serotonin and histamine in rabbit blood and small intestine 19 hours after the intravenous injection of either 0.1 mg/kg or 5 mg/kg of reserpine. The data reveal that the smaller amount of reserpine lowers blood serotonin and histamine to essentially the same level as the larger amount of the drug. However, 5 mg/kg of reserpine reduces the intestinal level for serotonin but not for histamine, whereas 0.1 mg/kg of reserpine does not lower the serotonin content of the intestines below the normal range.

Fig. 1 shows the increase in plasma levels for serotonin and histamine in a sensitized animal during anaphylaxis, following intravenous injection of 2 ml/kg of horse serum. The curves are similar to those shown previously(1). Unsensitized animals did not show an elevated plasma level for these amines after injection of the same amount of antigen.

Fig. 2 shows average plasma values for serotonin and histamine during anaphylaxis in rabbits that had been given 0.1 mg/kg of reserpine prior to injection of horse serum. The curves demonstrate that in comparison to Fig. 1, an increase in histamine still occurred. In contrast, serotonin levels were no longer elevated following reserpine.

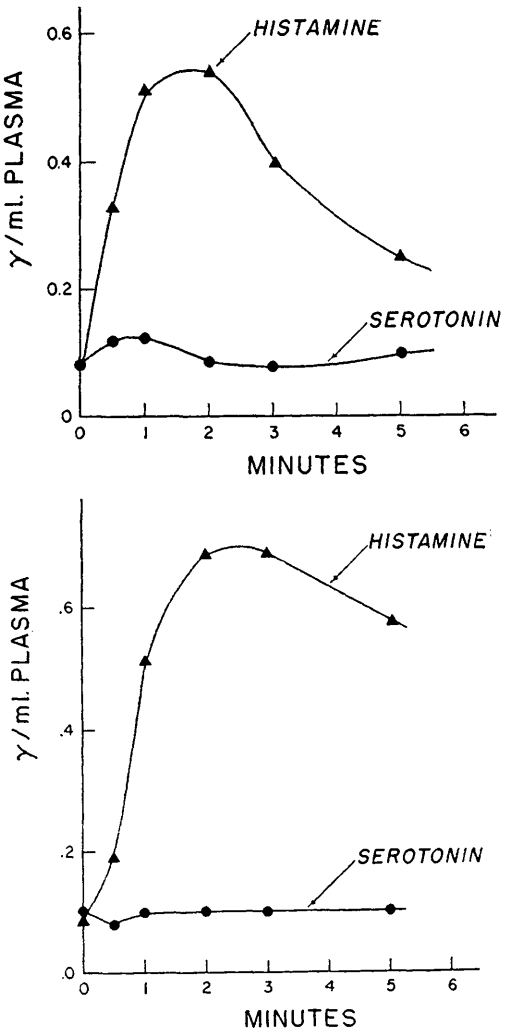

FIG. 2 (top). Plasma serotonin and histamine during anaphylaxis in rabbits that had received 0.1 mg/kg of reserpine 19 hr prior to inj. of the antigen (2 ml/kg of horse serum). Values presented are avg obtained from experiments on 3 animals.

FIG. 3 (bottom). Plasma serotonin and histamine during anaphylaxis in rabbits that had received 5 mg/kg of reserpine 19 hr prior to inj. of the antigen (2 ml/kg of horse serum). Values presented are avg obtained from experiments on 2 animals.

Fig. 3 gives average plasma values for serotonin and histamine during anaphylaxis in sensitized rabbits that had been given 5 mg/kg of reserpine before injection of horse serum. The curves are similar to those shown in Fig. 2.

Unsensitized animals, pretreated with reser-

pine in the same manner, did not show any increase in plasma concentration of serotonin and histamine after injection of horse serum.

Discussion. It appears that the major portion of the free serotonin found in rabbit plasma during anaphylaxis is probably released from platelets. On the other hand, appreciable amounts of histamine are liberated from tissues as well as from platelets following antigen-antibody interaction. However, despite these findings, it may well be that substantial amounts of serotonin could be liberated from tissues without the release being reflected by a rise in plasma serotonin. Previous work(1) showed that as much as 50 γ/kg of serotonin have been administered to normal rabbits by rapid intravenous injection without causing a rise in plasma concentration of this amine. In order to increase plasma levels of serotonin to those found during anaphylaxis, it was necessary to inject at least 150 γ/kg into a normal rabbit, whereas only 50 γ/kg of histamine were required to attain the levels found for this amine during anaphylaxis. These findings suggest that much more serotonin than histamine must be released during anaphylaxis to produce a comparable change in its plasma level, and that appreciable quantities of serotonin may be released from the tissues without producing a detectable rise in the plasma serotonin level.

Although most of the serotonin found in the rabbit is located in the intestinal tract, serotonin is also found in the lung (2-4 γ/g), and brain (0.4-0.7 γ/g). However, if the total amount of serotonin in these latter tissues were completely released during anaphylaxis, it is unlikely that the plasma serotonin would increase appreciably. Nevertheless, if only a fraction of the total lung and brain serotonin were released within these vital organs, the local effects could be profound. To consider that the relative importance of any substance, released during anaphylaxis, is demonstrated by the rise in its plasma concentration might be incorrect.

Further *in vivo* and *in vitro* tissue studies are being conducted with rabbits to clarify, if possible, the importance of the role of serotonin in anaphylaxis. In addition, similar studies are being conducted in other laboratory animals. In most animals, the serotonin content of platelets is much less than in rabbits. If future studies prove that serotonin is not released from tissues, but only from platelets, then the importance of this amine in anaphylaxis may be less in those species where the platelet content of serotonin is low.

Summary. 1. Reserpine injected intravenously into rabbits in the amount of 0.1 mg/kg causes a marked lowering of platelet serotonin and histamine without reducing serotonin or histamine in the intestinal tract below the normal range. 2. Sensitized rabbits, pretreated with 0.1 mg/kg of reserpine, had an elevated plasma level of histamine but not of serotonin during anaphylaxis. These findings suggest that the major portion of the rise in plasma serotonin during anaphylaxis is secondary to a release from platelets, whereas the histamine found in the plasma is probably released both from platelets and tissues. In rabbits that had been pretreated with 5 mg/kg of reserpine, histamine levels in the plasma during anaphylaxis were still elevated. 3. The significance of levels attained in the plasma as an index of release from tissues during anaphylaxis is discussed.

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