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ANAPHYLAXIS AND HISTAMINE RELEASE IN THE RABBIT

BY

M. SCHACHTER*

From the National Institute for Medical Research, Mill Hill, London, N.W.7

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Since the time that the similarity of the symptoms of histamine intoxication and acute anaphylactic shock was pointed out by Dale and Laidlaw (1910), an impressive body of evidence implicating histamine in anaphylaxis has accumulated. An increased concentration of circulating histamine was obtained by Dragstedt and Gebauer-Fuelnegg (1932) in supradiaphragmatic venous blood of the shocked dog. Similar observations were later made for the guinea-pig as well as for the dog (Code, 1939). The release of histamine from an isolated tissue during the antigen-antibody reaction of anaphylaxis was shown for the perfused guinea-pig lung (Bartosch, Feldberg, and Nagel, 1932), and Schild (1939), employing a tissue incubation technique, found that this was true, though to a varying degree, for many tissues of the guinea-pig. The anaphylactic release of histamine is marked in the liver of the dog (Dragstedt and Gebauer-Fuelnegg, 1932; Dragstedt and Mead, 1936; Rocha e Silva, Scroggie, Fidler, and Jaques, 1947), and, as has recently been demonstrated, also occurs in the isolated, perfused skin of this species (Feldberg and Schachter, 1952).

The above observations, along with others, support the hypothesis that the liberation of histamine is an important accompaniment of anaphylactic shock. The released histamine, through its effects at the site of release plus its subsequent systemic actions, can to some extent account for the toxicology of the immediate anaphylactic reaction in the guinea-pig and dog. Observations in other species, however, have raised some doubt about the "fundamental" role of histamine in anaphylaxis (see Ratner, 1943). For example, although histamine is released into the plasma from formed elements of rabbit blood during the antigen-antibody reaction *in vitro* (Katz, 1940; Dragstedt, Ramirez de Arellano, Lawton, and Youmans, 1940; Rose and Browne, 1941; Carryer and Code, 1950), in the intact rabbit, anaphylaxis is regularly accompanied by a marked reduction in the concentration of histamine in whole blood (Rose and Weil, 1939; Rose, 1941). Code and Hester (1939) likewise found a reduction in blood histamine concentration during the anaphylactic reaction in the horse and calf.

The present experiments were primarily designed to investigate whether direct evidence for the local release of histamine during the antigen-antibody reaction in various rabbit tissues could be obtained. They revealed that this does indeed occur, to a marked degree from skin, also from liver, and to a lesser degree from small intestine.

The possibility that other pharmacologically active substances might be released, or formed, during the anaphylactic reaction in the rabbit was also considered, since there is evidence that smooth muscle stimulating substances other than histamine may appear in the blood. For example, bradykinin, a substance producing a slow contraction of the guinea-pig ileum (Rocha e Silva, Beraldo, and Rosenfeld, 1949), has been shown to appear in the blood of dogs during anaphylaxis (Beraldo, 1950). The possibility that 5-hydroxytryptamine (serotonin, thrombotonin) might be released from platelets during the antigen-antibody reaction has been suggested by Rand and Reid (1952). Recent *in vitro* studies on blood using anti-platelet serum have revealed the release of a smooth muscle stimulating substance other than histamine or bradykinin (Moussatche and Cruz, 1952). Also, Humphrey and Jaques (1953) have reported the appearance of a substance resembling 5-hydroxytryptamine from rabbit blood during the antigen-antibody reaction *in vitro*. The present experiments, using the guinea-pig ileum as a test preparation, failed to detect the release of a smooth muscle stimulating substance other than histamine during the antigen-antibody reaction in isolated perfused skin, liver, or intestine.

MacIntosh and Paton (1949) commented on the similarity of anaphylaxis and the result of administration of histamine releasing substances, and

*Present address: The Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1

Mongar and Schild (1952) have since demonstrated a parallelism in the relative effectiveness of histamine releasing compounds such as 48/80 and curare with that of antigen in releasing histamine from guinea-pig tissues. These observations are of interest in so far as they suggest that a histamine releaser may be produced by the antigen-antibody reaction. The present experiments, however, demonstrate a discrepancy in the histamine releasing ability of these agents in the perfused rabbit liver; large amounts of 48/80 were ineffective, whereas antigen regularly released considerable amounts of histamine from sensitized preparations.

METHODS

White New Zealand rabbits (2.5–4 kg.) were sensitized by 6 daily intraperitoneal injections of 1 ml. horse serum and used for experiment in from 10 days to 6 weeks after the last injection. Anaesthesia was accomplished by intravenous sodium pentobarbitone (30 mg./kg.) plus ether if necessary. Arterial blood pressure was recorded from the carotid artery. Blood samples for plasma histamine assay were obtained in the following manner. Blood was drawn into a heparinized syringe with as little suction as possible and gently introduced into centrifuge tubes containing heparin. In later experiments syringes and centrifuge tubes were siliconed to reduce further the degree of "spontaneous" histamine release from the blood cells (see Code, 1952). The blood was immediately centrifuged at 3,000 rev./min. for 10 minutes, the plasma removed and assayed at once. In the dye experiments, a 5% solution of pontamine sky blue in saline was injected in a dose of 1 ml./kg., somewhat less than that used by Miles and Miles (1952) in studying permeability changes in guinea-pigs.

Perfusion of Isolated Skin.—The entire ear was removed after cutting away excess fur, and clipped on to a sloping square piece of glass. The perfusion system consisted of one or two elevated reservoirs containing the solutions to be perfused, which were continually aerated with 95% oxygen and 5% carbon dioxide. Polythene tubing led from the reservoir to a Murphy drip which permitted observation of the rate of inflow. Another long strip of polythene tubing connected the Murphy drip to a glass T tube. A short piece of rubber pressure tubing connected the T tube to the arterial cannula, a blunted hypodermic needle. Care was taken to remove air bubbles from the system, and the needle cannula was inserted into the central artery of the ear. After the flow was started the cut ends of the small veins of the ear were again cut with fine scissors to assist the flow and expel small clots. Single intra-arterial injections of 48/80 were made through the rubber tubing and the injected material was allowed to remain in contact with the ear tissue for 10–20 sec. by clamping off the inflow from the reservoir for this interval. The side arm of the glass T tube served as an air trap from which small bubbles could be removed if necessary. The perfusate ran along the margin of the supporting glass plate and was collected as it dropped. The rate of flow was regulated by a screw clamp on the polythene tubing so that it ranged from 18 to 25 ml./min. In those instances where the perfusion was altered from Locke solution to antigen (2% horse serum in Locke solution), two reservoirs were required and a glass Y tube was inserted in the system near the T tube (towards the reservoir). By opening the arm of the T tube the dead space fluid could be quickly removed on transferring from Locke solution to antigen. At the same time the polythene tubing connected to the Locke solution reservoir was completely clamped off. During the experiment the temperature of the skin of the ear was kept at 30–35° C. by a lamp. The fluid in the reservoir was usually kept at room temperature; in a few instances it was maintained at 35–40° C.

Perfusion of the Isolated Liver.—The arrangement of reservoirs, etc., was as for the ear perfusion. The liver, however, was perfused *in situ* and a glass instead of a needle cannula inserted into the portal vein. The abdomen was opened by a long mid-line incision, the intestine wrapped in a warm damp towel and pushed to one side. The portal vein was freed from the surrounding tissue and loose ligatures placed around it in preparation for cannulation. Small veins entering the portal vein superior to the loose ligatures were ligated. The chest was opened in the mid-line, retracted, and the thoracic inferior vena cava freed and prepared for cannulation. Artificial ventilation was begun after the chest was opened. The abdominal inferior vena cava was tied off above the right renal vein. The portal vein was then cannulated and perfusion begun. The thoracic aorta was quickly tied off and the inferior vena cava in the chest cannulated. The perfusate soon became clear and collection was begun. An abdominal pocket was built up around the liver with cotton-wool and the liver was immersed in warm paraffin which was kept at 35–40° C. by means of a lamp. The outflow was regulated to range between 25 and 35 ml./min.

Perfusion of Isolated Intestine.—A strip of upper intestine 18–30 in. in length (measured with mesentery removed after the experiment) was separated from the rest of the intestinal tract; its mesentery and blood supply were kept intact, and the superior mesenteric artery and portal vein were dissected free from surrounding tissue. The remainder of the intestinal tract was removed. The isolated loop of intestine was allowed 15–30 min. to recover from the operative procedure. Subsequently, glass cannulae were inserted into the mesenteric artery and portal vein, and the intestinal preparation was removed from the animal and transferred to a funnel-shaped bath maintained at 37° C. by a water jacket. The outflow of perfusate was regulated to 15–25 ml./min. In other respects the perfusion apparatus was the same as that used for skin and liver.

Histamine Assay.—This was performed on the isolated atropinized guinea-pig ileum preparation in an 18-ml. bath of magnesium-free Tyrode solution. 0.1 μg. atropine was added to the bath after each test. Abolition of the contraction by similar addition of 0.4 μg. of the antihistamine drug mepyramine served to identify the active substance as histamine. Those perfusates which contained 2% horse serum were assayed against histamine made up in Locke solution containing this concentration of horse serum, and equal volumes of the histamine standard and test solutions were added to the bath during assay. This precaution was taken, since histamine in 2% horse serum produced a smaller contraction than the same concentration of histamine in saline or Locke solution.

Histamine Extraction from Rabbit Ear.—The entire skin of the ear was easily stripped from the cartilage, weighed, finely divided with scissors and the histamine extracted by grinding with sand and hydrochloric acid as described by Douglas, Feldberg, Paton, and Schachter (1951). Since the cartilage contained very little histamine (<1.0 μg./g.), the histamine released into the perfusate can be considered as originating almost entirely from skin.

Drugs.—Histamine was used as the acid phosphate, atropine as sulphate, and mepyramine as maleate. Compound 48/80, a condensation product of *p*-methoxyphenethyl-methylamine with formaldehyde (Baltzly, Buck, de Beer, and Webb, 1949), was employed as a histamine liberator because of its relatively high potency in this respect (Paton, 1951). 5-Hydroxytryptamine was in the form of the creatinine sulphate and was kindly supplied by Dr. R. K. Richards of Abbott Laboratories, Chicago. Since serotonin (or thrombotonin) has been identified as 5-hydroxytryptamine (Rapport, 1949) the chemical term is employed for this substance. All values for histamine and 5-hydroxytryptamine are expressed as base.

RESULTS

Release of Histamine from the Skin of the Ear.—Experiments were performed on ear preparations from five sensitized rabbits and five similar experiments were carried out on ears from normal rabbits. The perfusion was started with Locke solution and was transferred to antigen (2% horse serum in Locke) after one or two 10 minute samples of perfusate had been collected. During perfusion with Locke solution the concentration of histamine in the perfusate from ear preparations of normal or sensitized rabbits was never greater than 0.01 μg./ml., and became undetectable after 10 minutes. Similarly, antigen failed to release histamine from ears of normal rabbits. In preparations from sensitized rabbits, however, perfusion with antigen resulted in the release of large amounts of histamine in every instance. The total amounts of histamine released in these experiments ranged from 22.8 to 41.5 μg., the detailed results of which are shown in Table I. The time course of the release in a typical experiment is shown in Fig. 1. Fig. 2 is a record of actual contractions of the guinea-pig ileum produced by successive perfusate samples before and during perfusion with antigen. It also shows the complete abolition of the activity of the samples by mepyramine.

TABLE I

THE RELEASE OF HISTAMINE FROM ISOLATED EARS OF SENSITIZED RABBITS ON PERFUSION WITH ANTIGEN (2% HORSE SERUM)

Exp. No.	Total Histamine in Perfusate from "Shocked" Ear (μg.)	Total Histamine Residue in "Shocked" Ear (μg.)	Total Histamine Released + Residue in "Shocked" Ear (μg.)	Total Histamine Extracted from Unperfused Ear (μg.)
1	34·7	89·6	124·3	132·0
2	22·8	96·0	118·8	131·2
3	34·9	68·9	103·8	127·3
4	41·5	177·8	219·3	216·0
5	37·4	92·5	129·9	144·6

In the experiments on ears from sensitized rabbits the histamine remaining in the skin of the ear was extracted after perfusion with antigen. Since the total histamine released into the perfusate was known it was possible to compare the total extractable histamine from the unperfused ear

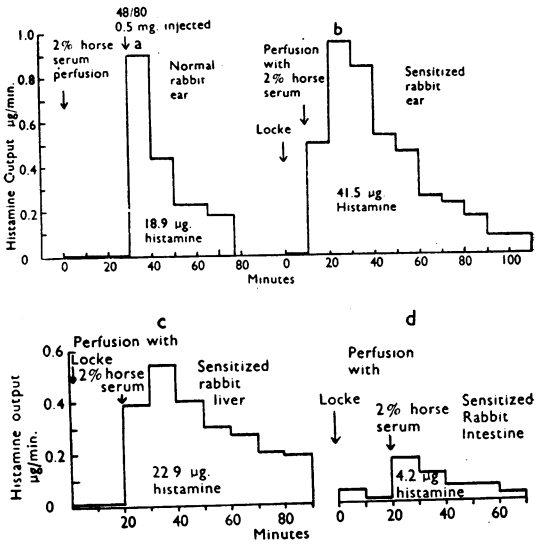

FIG. 1.—(*a*) Output of histamine from isolated ear of normal rabbit following a single intra-arterial injection of 0.5 mg. 48/80. The perfusion fluid in this experiment is 2% horse serum, which is completely ineffective in releasing histamine from the ear of a non-sensitized rabbit. (*b*, *c*, *d*) Output of histamine from isolated ear, liver, and intestine of sensitized rabbits on transferring perfusion from Locke solution to 2% horse serum (antigen).

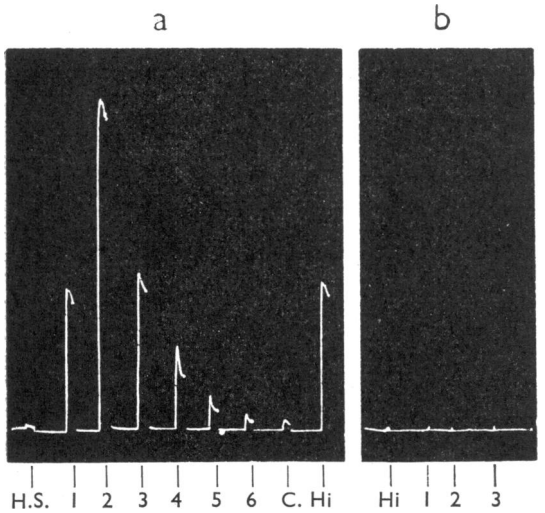

FIG. 2.—Contractions (15 seconds) of isolated guinea-pig ileum produced by perfusates from ear of sensitized rabbit obtained before and during perfusion with 2% horse serum (antigen). Between (*a*) and (*b*) 0.4 μg. mepyramine added to bath. (*a*) H.S., 0.5 ml. 2% horse serum. C., control, 1.0 ml. Locke perfusate before perfusion with horse serum. Hi, 0.1 μg. histamine in 2% horse serum. 1–6, 0.25 ml. perfusate of successive 10-minute samples during perfusion with 2% horse serum. (*b*) Hi, 0.2 μg. histamine. 1–3, 1.0 ml. of samples 1, 2, and 3 as in (*a*).

with the sum of that released from the "shocked" ear plus the amount subsequently extractable from it. The results indicate that the amount of histamine released from the skin of the rabbit's ear by antigen plus that remaining after the reaction corresponds to the total extractable histamine (Table I). The latter was, in most instances, slightly greater. This is probably due to some destruction of histamine during its release and subsequent collection. A similar conclusion regarding the pre-formed state of histamine released by antigen was deduced by Bartosch (1935) from experiments on perfused guinea-pig lung.

Single intra-arterial injections of 0.05–2.0 mg. compound 48/80 in 0.5 ml. saline regularly released histamine from Locke-perfused ears of normal rabbits. Repeated injections of the same amount of 48/80 released only small additional amounts. The amounts of histamine released in eleven experiments are shown in Table II. Except for a single experiment, where the injection of 0.5 mg. 48/80 released 82.0 μg. histamine, perfusion with antigen was more effective than 48/80 in releasing histamine. This is a reversal of the relative effectiveness of these two means of releasing histamine from dog skin (Feldberg and Schachter, 1952). As in the dog, however, the release by antigen was slower in onset and more protracted than by 48/80, and small amounts of histamine were still being released at a low but maintained concentration even after two hours of perfusion with antigen (Fig. 1).

TABLE II

HISTAMINE RELEASE ON INTRA-ARTERIAL INJECTION OF COMPOUND 48/80 IN THE ISOLATED PERFUSED RABBIT EAR

Amount of 48/80 Injected (mg. in 0·5 ml.)	Total Histamine in Perfusate (μg.)		
2·0	15·1		
1·0 (3)	13·5	12·1	9·7
0·5 (3)	82·0	18·9	16·3
0·1 (2)	11·0	9·5	
0·05 (2)	11·4	5·8	

The number of experiments is indicated in brackets.

The concentration of histamine in the whole skin of rabbit ear ranged from 16 to 30 μg. per g. in five ears (mean 23.2).

Release of Histamine from Liver.—The liver perfusate, unlike that of the ear, occasionally contained moderate amounts of histamine even when perfused with Locke solution. This varied between 0.01 and 0.07 μg./ml. and usually, though not always, fell to very low concentrations after perfusing for some time. However, transferring the perfusion from Locke's solution to 2% horse serum resulted in a prolonged increase in the output of histamine in six of seven experiments on livers from sensitized animals. The average total output of histamine by antigen in these six experiments was 22.4 μg. (S.D. 14.6). The mean histamine concentration in the perfusate for 20 minutes prior to antigen perfusion was 0.03 μg./ml., and 0.11 in the first 20 minutes during perfusion. A typical result is shown in Fig. 1.

Similar control experiments were done on seven normal preparations. In two of these experiments transferring the perfusion from Locke solution to 2% horse serum produced a release of histamine similar to that occurring in sensitized preparations. Rabbit liver thus occasionally shows a primary release of histamine on perfusion with horse serum. However, unlike the primary histamine release regularly evoked by horse serum from cat skin (Feldberg and Schachter, 1952) or by egg white in the intact cat and rat (Schachter and Talesnik, 1952), it was only occasionally observed in rabbit liver, and never from skin or intestine. Furthermore, it is not due to an occasional general tissue sensitivity, since horse serum perfusion of ears from the same two rabbits failed to release histamine.

Compound 48/80 was injected intraportally in four similar experiments. Injections of 1.0–5.0 mg. were either completely ineffective or produced only

traces of histamine in the effluent, irrespective of whether the perfusion fluid was Locke or horse serum solution. The ineffectiveness of 48/80 contrasts with the ready release of histamine by antigen from this organ of sensitized animals.

Release of Histamine from Intestine.—The basal histamine release from intestine was low, 0.01 μg./ml. or less, and rapidly became undetectable. In three control experiments on intestine from normal rabbits, perfusion with Locke or antigen solution failed to release any histamine or to augment the intestinal movements. In four of five experiments with intestine from sensitized rabbits, transfer of perfusion from Locke to antigen resulted in the release of definite though small amounts of histamine, giving totals of 0.2, 0.5, 1.0, and 3.8 μg. Increased intestinal movements always occurred with this release of histamine.

In three experiments 1 mg. 48/80 (0.05 ml.) was injected intra-arterially. This relatively large amount released no histamine or only traces of it.

Identification of Histamine in Perfusates.—Since Rand and Reid (1952) emphasized that 5-hydroxytryptamine (serotonin, thrombotonin) is present in considerable amounts in rabbit serum and may be readily mistaken for histamine if assayed on guinea-pig intestine, precautions were taken to ensure that the activity of the perfusates was in fact not partially due to the former substance. This was excluded by testing the effect of concentrations of mepyramine (0.4 μg. added to the bath) capable of abolishing the effect of histamine, against contractions elicited by 5-hydroxytryptamine. This had little or no effect on contractions produced by 5-hydroxytryptamine. Sensitive preparations of guinea-pig ileum treated with mepyramine could detect as little as 0.05 μg. and occasionally less of this substance. None the less, the activity of the perfusates was abolished by mepyramine even if two to three times the volume of perfusate assayed for histamine was tested. Therefore, the quantitative assay of the perfusates as histamine could not have included any activity due to 5-hydroxytryptamine.

Experiments with Pontamine Blue Dye.—Six normal rabbits were injected with a 5% solution of pontamine blue in saline by a marginal ear vein (1 ml./kg.). This was followed in 10 minutes by intravenous injection of 48/80 (1 mg./kg.) in two of the rabbits and by horse serum in another two. The remaining two received no further treatment. Thirty minutes after injection of dye all animals were killed by a blow on the head and a skin patch off the flank shaved, depilated, and removed. Three sensitized animals were likewise injected with dye and antigen and treated similarly. The normal animals all showed only faint blueness, and those which received horse serum or 48/80 after the dye showed no greater blueness than those receiving dye alone.

The three sensitized animals which were shocked by antigen, however, all showed markedly increased blueness of the skin, the degree of which appeared to be related to the severity of shock. The increased blueness of the skin was general and became evident in a few minutes. Miles (1953) has also noted widespread blueness of the skin during anaphylactic shock in guinea-pigs. Examination of the viscera revealed a markedly increased staining of the entire gastro-intestinal tract involving all layers of the gut wall. It was difficult to assess the degree of blueness of such normally dark organs as liver and spleen. There was no gross blueness of the lung. It is of interest that the intravenous injection of histamine (100–300 μg./kg.) did not increase permeability to circulating dye. A dose of 300 μg./kg. histamine administered intravenously in 30–60 sec. was found to be fatal in three rabbits under pentobarbitone anaesthesia and in three of four unanaesthetized animals.

A comparison of patches of skin removed from the flanks of normal dyed animals which received horse serum or 48/80 with those of sensitized animals following injection of antigen is shown in Fig. 3.

Plasma Histamine Concentrations in the Normal and Anaphylactic Rabbit.—Blood samples were taken from the femoral artery of normal rabbits

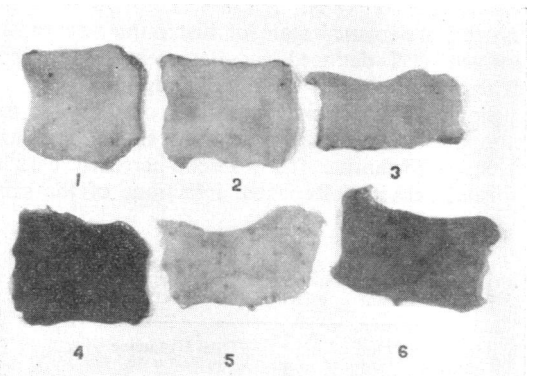

FIG. 3.—Patches of skin removed from the flanks of normal animals (1–3) which received (1) dye alone, (2) dye+1 ml./kg. horse serum, (3) dye+1 mg./kg. 48/80. The three animals show no difference in degree of blueness. (4), (5), and (6) are similar patches removed from three dyed animals in shock following 1 mg./kg. horse serum. Tissue (4) is from an animal which underwent severe shock, whereas (5) is from one which had a mild reaction. All substances were administered intravenously.

before and 1½–2 minutes after injections of horse serum (1 ml./kg.) or 48/80 (1 or 2 mg./kg.), and similarly from sensitized animals before and after serum. Centrifugation was immediately carried out and assay of the plasma histamine completed in about 20 minutes after withdrawal of blood. It was observed, as reported for rabbit serum by Rand and Reid (1952), that occasionally the contraction of the guinea-pig ileum produced by rabbit plasma was not entirely due to histamine, since it partially persisted after the preparation was rendered insensitive to histamine by mepyramine. However, mepyramine resistant activity was not detectable in volumes of plasma containing 0.04 μg. histamine or less. Hence the assay of small amounts of plasma (diluted 2 to 10 times if necessary) excluded the effect which was not due to histamine. By testing larger amounts of plasma (0.5–1.0 ml.) it was possible to study changes in the concentration of its mepyramine resistant activity. It is apparent, however, that rabbit plasma which contains several smooth muscle stimulants, and possibly inhibitory substances like adrenaline after shock, may be difficult to assay precisely for histamine. Hence the plasma histamine concentrations must be regarded as approximate.

Horse serum or 48/80 failed to produce significant increases in plasma histamine concentration in normal animals. In previously sensitized animals, however, horse serum produced increased plasma histamine concentrations in eight of nine experiments. The greatest increases occurred in two rabbits which died of shock in less than five minutes. The results were the same with or without siliconed glassware, but the high histamine values occasionally obtained in normal blood did not occur when this added precaution against "spontaneous" histamine release was taken. These results are shown in Table III.

Smooth Muscle Contracting Effect of Rabbit Plasma not Due to Histamine.—The occasional action of plasma (0.5–1.0 ml.) in causing a contraction of the guinea-pig ileum in the presence of mepyramine was also studied. This usually consisted of an immediate rapid contraction followed in about 20 seconds by a superimposed slow one which reached its maximum in approximately 60 seconds. This effect is probably in part due to 5-hydroxytryptamine, as the work of Rand and Reid (1952) on rabbit serum indicates. However, it was only partially reduced by tryptamine desensitization (Gaddum, 1953) of the guinea-pig ileum in the presence of mepyramine, which indicates that it cannot be entirely due to 5-hydroxytryptamine or similar compounds. The slow contracting component persisted after tryptamine desensitization.

Comparison of this activity of plasma obtained from normal animals before and after intravenous injection of horse serum or 48/80 indicated that its concentration paralleled the variations in the

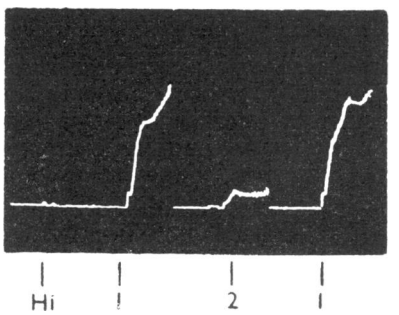

FIG. 4.—Effect of rabbit plasma, obtained before and during anaphylactic shock, on the guinea-pig ileum treated with mepyramine. Contraction time, 60 seconds. Hi, 0.5 μg. histamine. 1, 0.5 ml. plasma before shock. 2, 0.5 ml. plasma 2 minutes after injection of antigen.

TABLE III

PLASMA HISTAMINE CONCENTRATIONS (μg./ml.) IN NORMAL RABBITS BEFORE AND AFTER HORSE SERUM (1 ml./kg.) OR 48/80 (2 mg./kg.) AND IN SENSITIZED RABBITS BEFORE AND 1.5–2.0 MINUTES AFTER THE INTRAVENOUS INJECTION OF HORSE SERUM (1 ml./kg.)

Normal Rabbits						Sensitized Rabbits			
48/80			Horse Serum			Horse Serum			
Exp. No.	Before Injection	After Injection	Exp. No.	Before Injection	After Injection	Exp. No.	Before Injection	After Injection	Severity of Shock (Effect on Arterial B.P.)
1†	0·05	0·05	1	0·05	0·08	1	0·12	0·25	Not recorded
2	0·04	0·05	2	0·34	0·26	2	0·12	0·75	"
3	0·20	0·08	3	0·16	0·33	3	0·20	1·80	Very severe; dead in 5 min.
4*	0·05	0·05	4	0·05	0·03	4	0·32	1·20	" " " "
			5	0·02	0·01	5	0·34	1·0	Moderate; considerable recovery in 20 min.
			6*	0·13	0·17	6	0·60	0·50	"
			7*	0·06	0·07	7*	0·12	0·60	Severe; dead in 35 min. " "
						8*	0·08	0·20	Severe; dead in 60 min.
						9*	0·10	0·22	Moderate; considerable recovery in 20 min.

* All glassware siliconed. † 1 mg./kg. 48/80 injected.

histamine concentration. In sensitized animals, however, there was regularly a reduction of this activity 1½–2 minutes after intravenous injection of antigen, even when elevation of the plasma histamine concentration occurred. Fig. 4 illustrates a typical experiment; it shows the reduced contraction of the guinea-pig ileum in the presence of mepyramine, caused by rabbit plasma which was obtained during severe anaphylactic shock, when compared with a control sample of plasma from the same animal. In this experiment the plasma histamine concentration had risen at the same time from 0.34 to 1.0 μg./ml.

Discussion

The demonstration in the present experiments of the release of considerable amounts of histamine from isolated rabbit organs during the antigen-antibody reaction indicates that the release of histamine contributes to the toxicology of the anaphylactic reaction in this species. Its role in rabbit anaphylaxis is, therefore, qualitatively similar to that in the guinea-pig and dog. It is furthermore worth noting that the effect of the local release of histamine within specific organs may be more drastic than that due to histamine injected into the blood stream. For example, in the unanaesthetized dog the response to histamine release from skin is accompanied by severe cutaneous reactions, such as erythema, pruritus, and oedema, although no such effects occur when the amounts of histamine released by the whole animal are administered systemically (Paton and Schachter, 1951). Also, in the present experiments the intravenous injection of histamine in amounts up to the lethal dose failed to increase capillary permeability as evidenced by the degree of staining of tissues in the presence of circulating pontamine blue. However, even in non-lethal anaphylaxis there was a marked increase in blueness of the skin and gastro-intestinal tract.

The decrease in histamine concentration of whole blood which occurs in anaphylaxis (Rose *et al.*, 1939, 1941), despite its release from tissues, may be associated with the simultaneous trapping of platelet thrombi in various organs which occurs in shock (Fidler and Waters, 1946). Since the amount of histamine in rabbit platelets accounts for most of the blood histamine in this species (see Code, 1952), such a removal of platelets from circulating blood might greatly reduce the whole blood histamine concentration. Various other mechanisms, for example, the increased serum histaminase activity which occurs in anaphylactic shock (Rose and Leger, 1952), may also be involved. It is of interest in this regard that injection of less effective histamine liberators such as bile salt and neoarsphenamine produces a reduction in plasma histamine concentration in the cat (Schachter, 1952). The significant fact is, however, that histamine is released from a variety of rabbit tissues during the antigen-antibody reaction. Also, in the present experiments, elevation of the plasma histamine concentration was demonstrated in most instances of severe anaphylactic reactions.

The present experiments do not permit a precise assessment of the contribution of histamine to the lethal anaphylactic reaction, but the total amounts released from isolated organs by antigen indicate that enough may be released in the intact animal to produce severe or fatal reactions if it were administered by the intravenous route. For example, the average amount released from the skin of one ear alone was 34.6 μg. Perfusion experiments also demonstrated considerable release from liver (mean 22.4 μg.) and small but definite amounts from intestine. Also, substantial amounts of histamine are known to be released from the formed elements of rabbit blood *in vitro*, resulting in a mean increase of 0.8 μg./ml. plasma (calculation based on results of Carryer and Code, 1950). Since the experiments with pontamine blue support the probability that the anaphylactic reaction occurs in the skin generally as well as in the entire gastro-intestinal tract, it seems likely that in severe reactions the total amount of histamine released approaches the demonstrated lethal intravenous dose of histamine—viz., 300 μg./kg.

Recent experiments have, under various conditions, demonstrated the release of smooth muscle stimulating substances other than histamine during the antigen-antibody reaction (see Introduction). Our experiments have failed to detect the release or formation of non-histamine smooth muscle stimulants in perfusates from perfused skin, liver, or intestine as tested on the guinea-pig ileum. The release or formation of significant amounts of such substances must, therefore, either require the presence of blood or originate therefrom. However, the non-histamine smooth muscle contracting activity of rabbit plasma was regularly decreased at the height of shock in the intact animal even when elevations of plasma histamine concentrations occurred. Although the present results emphasize the importance of the release of histamine in rabbit anaphylaxis, it is quite possible that other released or formed substances contribute to the total reaction, as for example the release of heparin in canine anaphylaxis (Jaques and Waters, 1941) accounts for the incoagulability of the blood.

The fact that compound 48/80 failed to release histamine from perfused rabbit liver, whereas antigen readily did so, may indicate a significant difference in the mechanism of histamine release by these two agents. It would be of interest to extend this comparison to the dog, where the liver plays such a prominent role in anaphylactic shock.

SUMMARY

1. In normal rabbits, horse serum failed to release histamine from perfused skin and intestine, but occasionally did so from liver. It had no effect on the arterial blood pressure, and failed to alter permeability to circulating pontamine blue dye or to increase the concentration of plasma histamine.

2. In sensitized rabbits horse serum released considerable amounts of histamine from perfused organs. The greatest release was obtained from the skin of the isolated ear, moderate release from liver, and least from small intestine. Increased permeability to circulating dye occurred in shocked animals as evidenced by markedly increased blueness of the skin and gastro-intestinal tract, and increased plasma histamine concentrations were demonstrated in most instances of fatal or severe shock. These observations indicate that histamine release is a significant factor in the toxicology of the acute anaphylactic reaction in the rabbit.

3. The amount of histamine released from the skin of the perfused ear during the antigen-antibody reaction, plus the amount subsequently remaining, corresponded to the total amount of histamine extractable from the untreated ear. It is concluded, therefore, that the histamine released during this reaction is not formed, but comes from a pre-existing store.

4. Rabbit plasma occasionally exhibited a non-histamine smooth muscle contracting activity which was only partially due to 5-hydroxytryptamine, since a slow contraction of the guinea-pig ileum persisted after tryptamine desensitization. This activity, when present, was reduced in samples of plasma taken two minutes after anaphylactic shock.

5. Antigen was more effective in releasing histamine from perfused rabbit organs than the histamine releasing substance, 48/80. This was particularly true for the perfused liver, where the injection of large amounts of 48/80 failed to release histamine.

6. Assay of perfusates from skin, liver, and intestine, tested on the guinea-pig ileum, failed to demonstrate the presence of smooth muscle stimulating substances other than histamine after infusion of antigen or injection of 48/80.

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