TISSUE REACTIONS TO ANAPHYLACTIC AND ANAPHYLACTOID STIMULI; PROTEOLYSIS AND RELEASE OF HISTAMINE AND HEPARIN*

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(Received for publication, June 2, 1954)

It was shown in 1947 (1) that addition of specific antigen to tissue slices from sensitized guinea pigs causes proteolysis. A similar phenomenon was also observed when the antigen was added to serum (2, 3). These and other observations have been used to formulate an hypothesis explaining the mechanism of anaphylactic and anaphylactoid reactions (4, 5). An important part of these reactions is the release of histamine and heparin. By attempting to correlate proteolysis with the liberation of histamine and heparin, the present paper brings further evidence of an enzymatic mechanism of the allergic reactions.

Methods

Guinea pig tissues were used in all experiments. Lung and liver slices were prepared as described previously (1, 6). For each series of experiments 4 to 6 guinea pigs were killed by exsanguination and their lungs or livers were pooled. The tissues were cut with fine scissors into slices measuring about 2 to 3 mm., then washed in saline, dried on filter paper, and divided into lots of 2.5 gm. each. Each of the lots was suspended in 25 ml. of saline, buffered to pH 7.4 (0.15 N phosphate in 0.15 M NaCl), and kept at 37.5°C. throughout the experiment. After addition of the antigen or the anaphylactoid agent samples of the suspending fluid were taken at intervals of 5 minutes: 1 ml. was added to 4 ml. of 15 per cent trichloracetic acid, 2 ml. was kept on ice for histamine or heparin estimation.

Homogenates of tissues in buffered saline were prepared by grinding up the slices in a Waring blendor, using a semimicro container. These were clarified by centrifuging the homogenates at 5700 *G* for 30 minutes and discarding the solid residue.

Lung perfusions were performed according to the technique described by Rocha e Silva (7) with a few slight modifications. After the thorax had been opened, the pulmonary artery was cannulated and the lungs were excised and placed in a moist chamber. They were perfused with Tyrode's solution maintained at 37.5°C., oxygenation being assured by artificial respiration of the isolated lung. Perfusion pressure was regulated to give a flow of 7 to 8 ml. per minute. At the beginning of the experiment, the preparation was perfused until no trace of color could be detected in the effluent. Thereafter, the effluent was collected

* This investigation was supported partly by the Office of Naval Research, United States Department of the Navy, under contract No. N. R. 114–161, and partly by the Atomic Energy Commission, under contract No. AT (11-1)-67.

from the open pulmonary veins by means of a funnel leading into graduated cylinders. The agents to be tested were added to the perfusion fluid from a side arm placed close to the cannula.

Protein breakdown was measured by the increase of ultraviolet absorption of the trichloracetic acid filtrate. The samples treated with trichloracetic acid were filtered, after at least 1 hour's standing, on Whatman No. 50 filter paper and read on the Beckman spectrophotometer at $\lambda = 280$ mμ against a blank containing all the reagents added to the suspension medium. In perfusion experiments correction of the readings for protein-containing material introduced into the perfusion fluid was done by means of the ratio between readings for total protein (without trichloracetic acid) and the non-precipitable fraction. This ratio being defined for the material injected, all increase in non-precipitable material could be interpreted as coming from the tissue.

Optical density readings are expressed in terms of milligrams of "protein hydrolyzed." This conversion is based on the assumption that increase in non-precipitable material absorbing at 280 mμ, under the conditions of the experiment, can only be caused by the breakdown of tissue proteins. Since the exact nature of these proteins is unknown the conversion was calculated by means of the the extinction coefficient of 0.9 (for 1 mg. of protein per ml.) which is an average value for the known tissue proteins. Although necessarily an approximation, this treatment reflects the right order of magnitude of the protein breakdown.

Tissue protease was prepared from the saline extracts described above. The extract was diluted 20-fold with distilled water and the pH was adjusted to 5.2. The resulting globulin precipitate, collected after centrifugation, was dissolved in buffered saline an enzyme activity tested according to the method previously described (3).

Histamine was estimated with the usual bio-assay method on guinea pig ileum. A 2 cm. long strip was suspended in 10 ml. of oxygenated Tyrode's solution maintained at 37.5°C. Contractions were recorded with a light lever. The heights of contractions caused by varying amounts of the diffusates and perfusates were matched against those caused by known amounts of histamine. Neo-antergan (at the concentration of 10^{-6}) inhibited the action of the samples. All results are expressed in terms of histamine base.

Heparin estimations were done by the method of Monkhouse and Jaques (8). 2 ml. of diffusate to be tested was mixed with 1 ml. of 80 per cent phenol and left overnight at room temperature. The mixture was then centrifuged for 20 minutes at 2500 R.P.M., the aqueous layer pipetted off and washed with ether to remove all the phenol. One ml. of the aqueous solution was mixed with 3 ml. of buffered saline and 1 ml. of a 0.012 per cent solution of azure A. The resulting metachromatic color, which remains constant for about 10 minutes, was read at 505 mμ in a Coleman universal spectrophotometer against a blank containing the same concentration of dye. When tested against standard solutions, the method gave proportional increases of metachromatic color up to about 25 μg. of heparin per sample. Whether the method measures only heparin in tissues or whether other substances affect the metachromatic reaction, is still undecided (8). Since, however, the release of heparin is a fact well established by a variety of methods (28, 29), the values obtained under the conditions of these experiments can be interpreted as due to heparin. Results are expressed in terms of weight of Na heparin,[1] used for standardization.

Two antigens were used for sensitization of guinea pigs: egg albumin[2] given in a single subcutaneous injection (5 mg.), and rabbit serum (0.5 ml.) given intracardially. The animals were used 20 to 30 days after sensitization.

[1] Kindly supplied by Abbott Laboratories, Chicago.

[2] Soluble egg albumin, Merck & Company, Inc., Rahway, New Jersey.

RESULTS

Tissue Slices.—When guinea pig lung slices were suspended in buffered saline and incubated at 37.5°C. for 20 minutes a certain degree of protein breakdown and histamine release was nearly always observed. It is assumed that these changes observed in the control samples were due to the trauma involved in the slicing of the tissue.

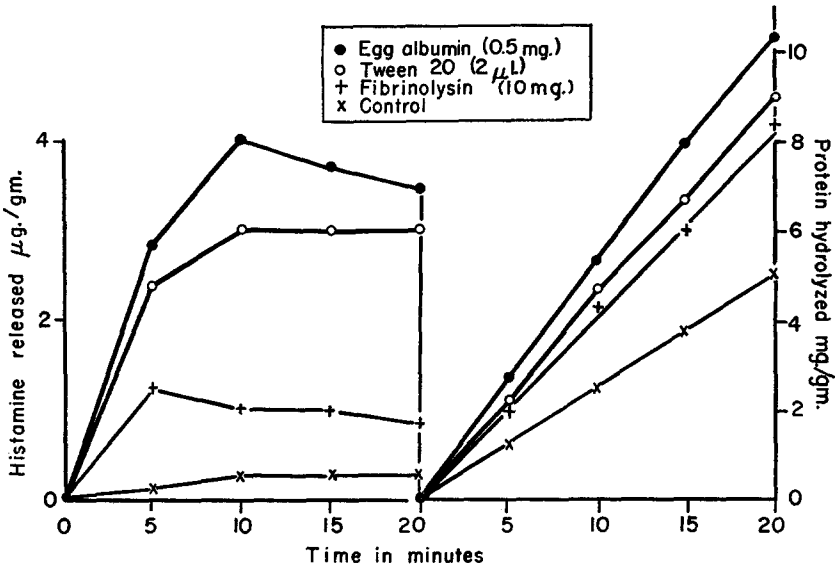

FIG. 1. Histamine release and proteolysis in guinea pig lung slices submitted to specific antigen, an anaphylactoid agent (tween 20), and fibrinolysin. Abscissa: time in minutes; ordinates: left, histamine released in micrograms per gram of tissue; right, protein hydrolyzed in milligrams per gram of tissue. The concentrations indicated are per milliliter of the suspending fluid.

Fig. 1 summarizes the results of a representative series of experiments: it shows the action of homologous antigen, of an anaphylactoid agent, tween 20 (9),[3] and of the protease fibrinolysin,[4] on protein breakdown and histamine release. It is seen that, while proteolysis proceeds at a steady rate, histamine release reaches a maximum between 5 and 10 minutes and does not increase further. The slight decrease observed sometimes after the initial release probably is due to a spontaneous or enzymatic destruction of histamine.

The most appropriate way to express the results of these experiments seemed to be in terms of the amounts of protein hydrolyzed and histamine released

[3] Batch 225 B, by courtesy of Atlas Powder Co.

[4] Bovine fibrinolysin (plasmin), kindly supplied by Dr. E. C. Loomis, Research Laboratories, Parke Davis and Company Detroit, Michigan.

per gram of tissue within the first 10 minutes, after subtracting the control values obtained for each pool of guinea pig lung.

It is seen in Table I that increasing concentrations of anaphylactic or anaphylactoid agents (tween 20, octadecylamine, morphine, and 48/80) cause more histamine to be released and more protein to be hydrolyzed. This is consistent, at least for histamine release, with the results of Mongar and Schild (10). It is also seen that all the agents do not elicit quantitatively the same reaction: 48/80[5] is more potent than morphine which is more active than octadecylamine which in turn is more active than tween 20. The last one has, in this experiment, approximately the same potency as the specific antigen.

TABLE I

Protein Breakdown and Histamine Release in Guinea Pig Lung Slices Submitted to Anaphylactic and Anaphylactoid Agents

Agent		Protein hydrolyzed*	Histamine released*
		mg./gm.	*μg./gm.*
Rabbit serum (sensitized lung)	0.002 ml.	0.3	0.5
	0.01 ml.	1.0	0.85
	0.1 ml.	1.7	1.9
Rabbit serum (normal lung)	0.1 ml.	0	0
Tween 20	0.2 μl.	0.4	1.0
	2.0 μl.	1.0	1.5
	20.0 μl.	1.2	1.75
Octadecylamine	0.16 mg.	1.0	1.0
	0.8 mg.	1.4	2.0
	1.2 mg	2.0	2.9
Morphine sulfate	0.5 mg.	1.6	1.5
	1.0 mg.	2.0	2.3
	1.5 mg.	2.45	2.5
48/80	0.1 mg.	2.0	2.0
	0.5 mg.	2.4	3.8
	1.0 mg.	2.6	4.3

In each sample 2.5 gm. of lung slices was suspended in 25 ml. of buffered saline (pH 7.4). Concentrations of the agents are expressed per milliliters of suspending medium. Values obtained with the untreated controls were subtracted in each case.

* Amounts obtained in the first 10 minutes of the experiment.

[5] Condensation product of N-methylhomoanislyamine with formaldehyde, resulting in the formation of a mixture of di-, tri-, and tetramers; kindly supplied by Dr. E. J. de Beer, Wellcome Research Laboratories, Tuckahoe, New York.

Differences in potency are nearly always the same for the two phenomena of histamine release and proteolysis.

This is made clearer in Fig. 2 in which all 44 tissue slice experiments are shown with histamine release plotted against protein breakdown. The best fitting lines were calculated by means of the least squares method for each of the four groups of experiments: antigen-

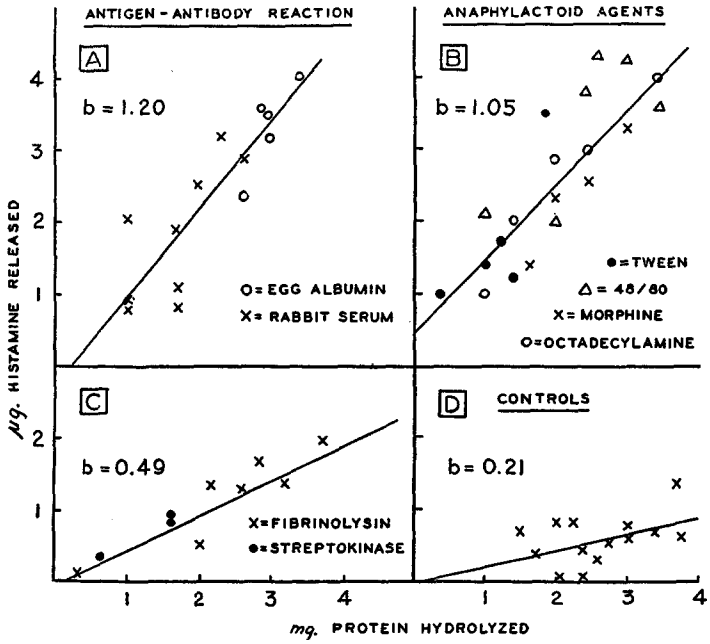

FIG. 2. Histamine release as a function of proteolysis in guinea pig lung slices submitted to the specific antigen (A), anaphylactoid agents (B), fibrinolysin or streptokinase (C), and without any treatment (D). Abscissa: milligrams protein hydrolyzed per gram of tissue; ordinate: micrograms histamine released per gram of tissue during the first 10 minutes. The slope of the lines (b) was calculated by means of the least squares method. The values plotted in A, B, and C are those obtained after subtraction of the corresponding controls.

antibody reaction (A), anaphylactoid agents (B), fibrinolysin and its direct activator streptokinase[6] (C), and the untreated control samples (D). In A and B the slopes are very close and the difference is well within the experimental error. It can be stated that, when anaphylactic or anaphylactoid agents are applied to the lung, for each mg. of protein hydrolyzed approximately 1.1 μg. of histamine is released in the first 10 minutes.

The fact, shown in group C, that fibrinolysin and streptokinase cause histamine to be released is an important argument in favor of a proteolytic mecha-

[6] Preparation of streptokinase and streptodornase (Varidase), Lederle Laboratories Division, Pearl River, New York.

nism being involved in histamine liberation. However, the slope obtained in plotting these experiments is significantly different (as ascertained by the *t* test) from the slopes in A and B. The correlation coefficient of all the experimental results represented in sections A, B, and C is 0.72 which was found to be highly significant when tested by Fisher's method (11). Correlation coefficients for the four groups were 0.64 for A, 0.72 for B, 0.81 for C, and 0.42 for D. The first three were found to be significant with Fisher's test.

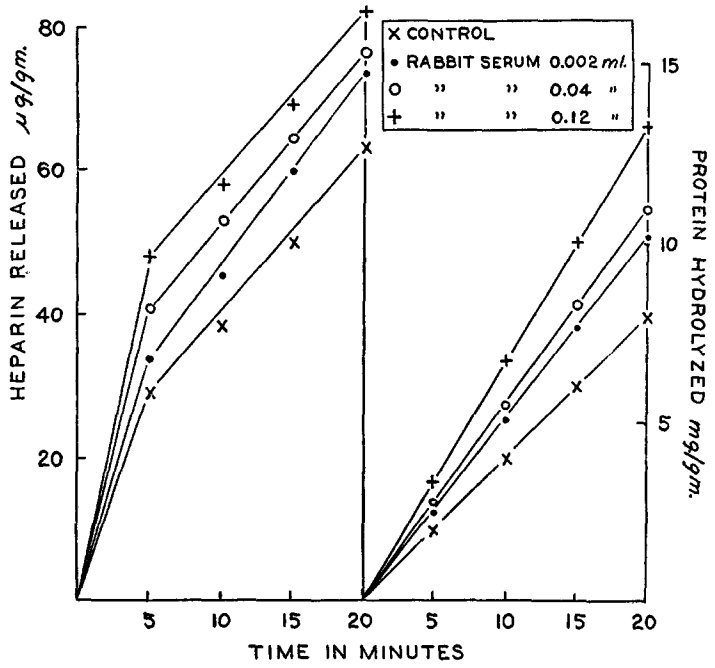

FIG. 3. Heparin release and proteolysis in guinea pig liver slices submitted to the specific antigen. Abscissa: time in minutes; ordinates: left, heparin released in micrograms per gram of tissue; right, protein hydrolyzed in milligrams per gram of tissue.

In eight experiments, histamine (in concentrations from 0.1 to 20 μg. per ml.) was added to lung slices. In no instance was the proteolytic reaction elicited and no release of histamine could be detected.

Experiments performed with liver slices also show that histamine release is not the cause of proteolysis. Although it is well known (30) that guinea pig liver does not release histamine, proteolysis could still be induced in this tissue just as well as in lung.

Fig. 3 shows the results of one experiment in which increasing concentrations of rabbit serum were added to liver slices taken from guinea pigs previously sensitized to this antigen. Fig. 3 also shows that, simultaneously with the breakdown of protein, a material giving

TABLE II

Protein Breakdown and Heparin Release in Guinea Pig Liver Slices Submitted to Anaphylactic and Anaphylactoid Agents

Agent		Protein hydrolyzed*	Heparin released*
		mg./gm.	*μg./gm.*
Rabbit serum (sensitized liver)	0.002 ml.	1.1	7.0
	0.04 ml.	1.5	15.0
	0.12 ml.	2.7	19.0
Egg albumin (sensitized liver)	5.0 mg.	1.3	8.5
Egg albumin (normal liver)	5.0 mg.	0	1.5
Tween 20	2 μl.	1.2	10.0
48/80	0.5 mg.	1.5	12.0
	1.0 mg.	1.7	14.0

In each sample 2.5 gm. of liver slices was suspended in 25 ml. of buffered saline (pH 7.4). Concentrations of the agents are expressed per milliliters of suspending medium. Values obtained with the untreated controls were subtracted in each case.

* Amounts obtained in the first 10 minutes of the experiment.

TABLE III

Inhibition of Protein Breakdown and Release of Histamine and Heparin in Lung Slices Submitted to Anaphylactic and Anaphylactoid Agents

Agent		Inhibitor		Protein hydrolyzed	Histamine released	Heparin released
		mg./ml.		*mg./g.*	*μg./g*	*μg./g*
Egg albumin	5 mg.	—		2.6	2.4	17.0
	5 mg.	Na salicylate	1.0	0.5	0.4	6.0
	5 mg.	SBTI*	0.1	0.3	0.1	0
	5 mg.	"	0.5	0	0	—
	5 mg.	Polylysine	1.0	0.3	0.1	3.0
Tween 20	2 μl.	—		1.2	1.75	12.0
	2 μl.	Na salicylate	1.0	0.2	0	2.5
	2 μl.	Polylysine	1.0	0.3	0	—
Octadecylamine	0.8 mg.	—		1.4	2.0	—
	0.8 mg.	Na salicylate	1.0	0.2	0.15	—
	0.8 mg.	SBTI*	0.1	0.3	0.1	—
	0.8 mg.	"	0.5	0	0	—

In each sample 4 gm. of lung slices was suspended in 40 ml. of buffered saline (pH 7.4). Concentrations of inhibitors and other agents are given per milliliters of suspending fluid. Values obtained with the controls were subtracted in each case; when inhibitors were used, these were also added to the control samples.

* SBTI, soy bean trypsin inhibitor.

the metachromatic reaction was released from the liver slices. It should be noted that the rabbit serum used did not contain any of this heparin-like material.

Table II summarizes the experiments performed with liver slices, giving the amounts of protein hydrolyzed and heparin released per gram of tissue in the first 10 minutes. The control values were subtracted from the experimental figures.

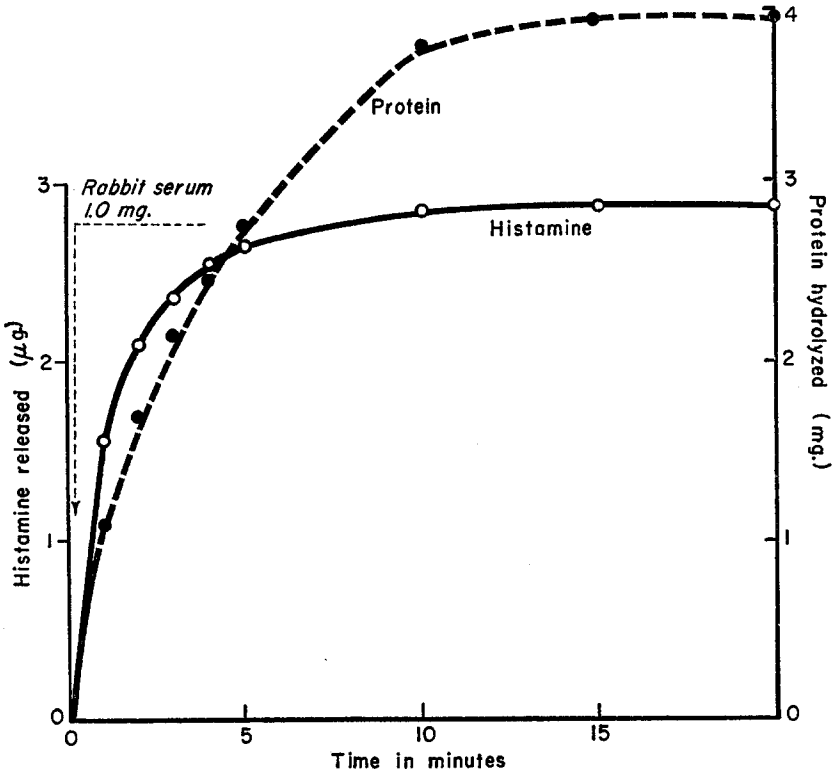

FIG. 4. Histamine release and proteolysis in perfused guinea pig lung. Abscissa: time in minutes; ordinates: left, histamine released (micrograms); right, protein hydrolyzed (milligrams) expressed in cumulative values. At the arrow, the specific antigen was introduced into the perfusion fluid close to the ingoing cannula.

Heparin-like material was also released from lung tissue in amounts comparable to those obtained from liver. In a number of experiments, performed with lung slices, the three processes of proteolysis, histamine liberation, and heparin release were studied simultaneously. The main purpose of these experiments was to find out whether inhibition of proteolysis will affect the release of histamine and heparin.

Among the compounds tested were sodium salicylate which was found to inhibit fibrinolysin *in vitro* (12), a crystalline protein isolated from soybean by Kunitz[7] (13) and which

[7] General Biochemicals, Inc., Chagrin Falls, Ohio.

has well known inhibitory properties for trypsin, fibrinolysin, and other proteases, and a polymer (31-mer) of lysine[8] which was previously found to prevent the conversion of profibrinolysin into fibrinolysin (3). The inhibitors were added to the suspension fluid at the concentrations indicated in Table III. The antigen and other agents were added a few minutes later and samples were taken as indicated above. It is seen in Table III that all three inhibitors tested depressed not only protein breakdown but also the release of histamine and heparin.

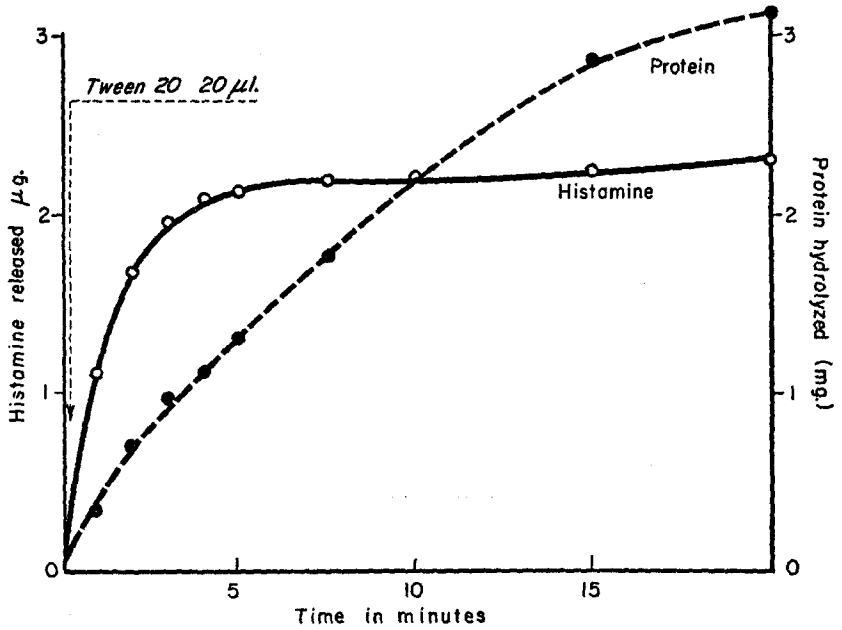

FIG. 5. Histamine release and proteolysis in perfused guinea pig lung. Abscissa: time in minutes; ordinates: left, histamine released (micrograms); right, protein hydrolyzed (milligrams) expressed in cumulative values. At the arrow, an anaphylactoid agent was introduced into the perfusion fluid close to the ingoing cannula.

Lung Perfusion.—Correlation between proteolysis and histamine release was also demonstrated under the more physiological conditions of a perfused lung preparation. It was shown that adding the specific antigen to the fluid perfusing a sensitized lung caused not only liberation of histamine—this has been known since the experiments of Bartosch, Feldberg, and Nagel (14)—but also the appearance of protein breakdown products in the effluent.

Fig. 4 shows the cumulative amounts of histamine liberated and protein hydrolyzed during the 20 minutes following the addition of antigen. Fig. 5 shows a similar experiment in which the anaphylactoid agent tween 20 was added to the fluid perfusing a normal guinea pig lung.

The perfusion experiments yielded results closely similar to those of the tissue slice ex-

[8] Kindly supplied by Dr. E. Katchalski, Weizmann Institute of Science, Jerusalem, Israel.

periments. In seven perfusions the mean ratio of μg. of histamine released per mg. of protein hydrolyzed was found to be 1.2 for the first 10 minutes. The main difference was in the rate of proteolysis which remained constant in the slices but declined in the perfused preparations. This may be explained by the fact that the activator and probably also the active enzyme were constantly washed out from the perfused tissue whereas they remained in the slices.

Another difference between perfusion and tissue slice experiments was the comparatively slow rate of proteolysis and histamine release in the perfused

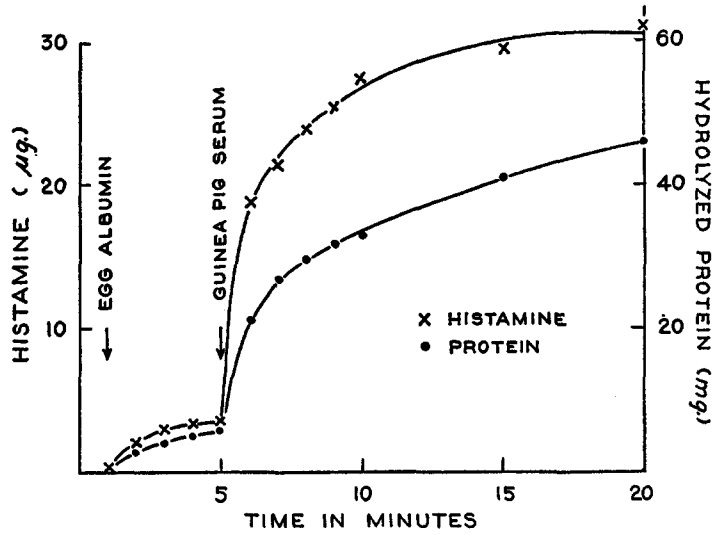

FIG. 6. Action of normal guinea pig serum on the release of histamine and proteolysis in perfused guinea pig lung. Abscissa: time in minutes; ordinates: left, histamine released (micrograms); right, protein hydrolyzed (milligrams) in cumulative values. At the first arrow perfusion was switched to antigen-containing fluid (supplying 0.15 mg. of egg albumin per min.). At the second arrrow 1 ml. of fresh, normal guinea pig serum was introduced into the perfusion fluid and 30 seconds later the antigen infusion was stopped by switching back to normal Tyrode's solution.

lung (average weight 2.5 gm.) as compared with the tissue slices. According to Rocha e Silva (7) presence of serum in the perfusion fluid increases the amount of histamine released.

In a few experiments normal guinea pig serum was added to the perfusion fluid. The results of one of these experiments are shown in Fig. 6. At the first arrow, the perfusion is switched for 5 minutes to a Tyrode's solution containing 20 μg. of egg albumin per ml. It is seen that the action on proteolysis and histamine release is similar to that shown in Fig. 4. At the second arrow, 30 seconds before switching back to plain Tyrode's solution, 1 ml. of fresh, normal guinea pig serum was injected into the perfusion cannula. In the absence of the antigen, such an injection does not produce any significant effect. In the present case, however, an immediate and very notable increase in the rate of histamine release and proteolysis was observed. Within 15 minutes after the injection of serum over 30 μg. of histamine

was liberated and over 40 mg. of protein hydrolyzed. The latter figure represents approximately 20 per cent of the total saline extractable proteins of the lung. Two similar experiments gave, on addition of serum, increases in rate of the same order.

Enzyme extraction.—

Amounts of 250 or 500 mg. of tissue slices were treated with the specific antigen, tween 20, morphine, or 48/80. After a few minutes contact, the mixture was homogenized in buffered

TABLE IV

Proteolytic Activity of Extracts of Tissues Treated with Anaphylactic and Anaphylactoid Agents

Tissue	Agent		Units per g. of tissue		
			Activator added to		
			Slices	Homogenate	Extract
Lung	Rabbit serum*	1.0 ml.	52.5	—	—
		1.0 ml.	36.0	19.0	2.5
		1.0 ml.	35.0	23.0	0.5
	Tween 20	0.2 ml.	33.5	13.0	0
	Morphine sulfate	0.75 mg.	29.0	12.0	0
	48/80	0.25 mg.	24.0	—	—
	Streptokinase	20,000 units	43.5	37.5	41.0
Liver	Rabbit serum*	0.5 ml.	40.0	8.0	1.0
		1.0 ml.	47.0	—	—
	Morphine sulfate	0.3 mg.	24.0	—	—
		0.75 mg.	28.0	—	—
		1.5 mg.	43.0	10.0	3.0
		3.0 mg.	75.0	—	—
	48/80	0.5 mg.	21.5	—	—
		1.0 mg.	30.0	—	—
		1.0 mg.	32.0	7.0	1.0

Concentrations of activators are expressed per gram of tissue.

* Added to tissues from guinea pigs sensitized to rabbit serum.

saline and centrifuged as described above. The globulin fraction was then tested for protease activity on fibrinogen as a substrate.

Results of these experiments are shown in Table IV (first column). It is seen that addition of the various agents to lung or liver slices caused an average of 37 units of protease activity to appear in the extract. This is over and above the spontaneous activity that may have been present in the untreated tissue, since the latter was used as a blank.

When the activating agents were added to tissue homogenates, extraction of the enzyme resulted in consistently lower protease activities (Table IV, fourth column). When the

activating agent was added to the tissue extracts free from cell debris, enzymatic activity fell to very low levels and in some cases was not detectable at all (Table IV, fifth column). The only exception was streptokinase which produced approximately the same enzyme activity in all three cases.

These observations suggest that protease activation in tissues requires the intervention of a kinase system. Such a requirement has been demonstrated for the activation of the protease precursor present in serum (3).

DISCUSSION

The results just described show a correlation between protein breakdown and liberation of histamine and heparin in tissues reacting to anaphylactic or anaphylactoid agents. Correlation, however, does not necessarily mean causal relation between phenomena. The sequence of biochemical events leading to the allergic reaction is still uncertain.

The explosive character of histamine release would naturally suggest that it is the first step. It was seen, however, that addition of histamine to tissue slices does not cause proteolysis and also that protein breakdown does take place in the guinea pig liver without being associated with histamine release.

The hypothesis according to which proteolysis is the causal event in anaphylaxis and related phenomena is an old one and its development has recently been reviewed (5). The evidence for the hypothesis can be summed up as follows: (*a*) proteases or protein breakdown products can be demonstrated in biological systems in which an antigen-antibody reaction or an equivalent anaphylactoid reaction occurs; (*b*) proteases, such as trypsin or fibrinolysin can release histamine when added to tissues *in vivo* or *in vitro*; (*c*) compounds known primarily as protease inhibitors inhibit also the liberation of histamine.

Understanding of the mechanism of histamine release is greatly hampered by the complete lack of knowledge of the bond which links histamine to some large non-diffusible molecule, presumably a protein. "Bound histamine" is an entirely hypothetical compound which has never been isolated or even shown to exist in cell-free systems. There is no convincing evidence indicating that histamine is bound to proteins by a peptide linkage, although the theoretical possibility of such a linkage has been demonstrated (15).

When the first synthetic "histamine liberators" were described (16), it was assumed that they acted by displacing histamine from a loose physical linkage (H bond) by which it was attached to protein molecules. However, this assumption became difficult to maintain when new histamine liberators were discovered which possessed no amine groups (tweens, dextran) and when it was shown that "histamine liberators" could release other substances, for example, heparin (17). There are three main possibilities compatible with the facts known today. The first is that the antigen-antibody reaction or its equivalent causes the appearance of a free proteolytic enzyme which, by breaking down

certain protein molecules, sets free histamine, heparin, and other substances attached to them. Such a mechanism does not necessarily require histamine to be attached with a peptide link. The second possibility is that the proteolytic process first sets free certain peptides or amino-acids which secondarily would displace histamine from a loose physical bond. The third possibility is that activation of the protease precursor and histamine release are produced by a common cause which may be the activity of the kinase system. That this kinase action may also be enzymatic seems to be supported by some recent observations (18, 19).

It should be mentioned that McIntire and his coworkers (20–22), using rabbit blood cells, were unable to release histamine by streptokinase or to prevent histamine release by soy bean trypsin inhibitor. The kinetic evidence they produced against an enzymatic mechanism for histamine liberation (22) could be interpreted as proving such a mechanism provided one can assume that it is constantly being controlled by an inhibitor. There is a great deal of evidence for the validity of such an assumption (23, 24). According to Code (25) rabbit blood is not suitable for histamine release studies.

The proteolytic enzyme system which, in guinea pig serum, is activated by anaphylactic and anaphylactoid agents has been identified with the fibrinolytic system (3). This identification, however, can only be a tentative one since the fibrinolytic system is as yet poorly known and probably includes several proteases. There is no valid ground for a useful discussion about identifying the tissue protease described in the present paper with the serum protease. Evidence, illustrated in Fig. 2, suggests a difference between the guinea pig lung protease and fibrinolysin prepared from bovine plasma. The only character common to proteases which may play a role in allergic phenomena and to fibrinolysin is that they exist in tissues and body fluids as inactive precursors. These precursors are converted into the active enzyme by the agents studied in the present report.

The kinase which brings about the conversion of the proenzyme seems to be different in the serum and tissue systems. In guinea pig serum this kinase was shown to be indistinguishable from complement (3). Repeated attempts were made to determine complement in lung tissue by using slices, homogenates, or extracts in a hemolytic system but only traces could be detected (2 to 5 units per gm.). Even these small amounts dwindled to a maximum of 0.5 units per gm. when the lung was washed by perfusion prior to the slicing. It is therefore probable that tissue kinase is different from serum kinase; it may be identical with the cytofibrinokinase described by Astrup (26) and Tagnon and Palade (27).

The perfusion experiments illustrated in Fig. 6 suggest that serum kinase can also activate the tissue proenzyme and that this activation produces even better results. One can assume that in the intact animal both serum and tissue

kinases play a role. Conditions as they exist *in vivo* are more closely approximated when serum is added to the antigen than in the case of the tissue being suspended in or perfused by a purely artificial medium.

SUMMARY

Addition of the specific antigen to slices of liver or lung taken from sensitized guinea pigs, or the addition of anaphylactoid agents (tween 20, octadecylamine, morphine, and 48/80) to tissue slices from normal animals, or the perfusion of lung with these agents, has been shown to cause protein breakdown and liberation of histamine and heparin. The close correlation between these phenomena raises the question of which is the causal event. Suppression of histamine and heparin release by inhibition of proteolysis suggests that the latter is the more fundamental reaction, but the problem probably can not be decided on the basis of present knowledge.

Tissue proteolysis induced by the agents investigated in this work results from the action of a protease present in normal tissues as an inactive precursor. Conversion of the proenzyme requires the intervention of a kinase. The tissue kinase seems to be different from the serum kinase which has been shown to be related to complement. Serum kinase, however, also acts on tissue proenzyme and probably plays an important role in tissue reactions as elicited in the intact animal.

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