STUDIES ON THE PHARMACOLOGY OF HISTAMINE RELEASE

by

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PART 1. INTRODUCTION

A. A Brief History of Histamine Research

When Windaus and Vogt first synthesized histamine in 1907, the story of histamine release had already begun. Twenty-seven years earlier Schmidt-Mulheim (1880) had described a profound state of shock occurring in the dog directly after the intravenous injection of the proteose-peptone mixture known as "Witte's peptone". The mechanisms underlying the events he recorded were not to be known until almost 60 years later. Even to-day the phenomenon of "peptone shock" remains a partial mystery and the actual beginnings of the reaction which culminates in the release of histamine are virtually unknown.

Many of the early workers seem to have been hampered by the confusion arising from the multitudinous descriptions of pharmacologically active substances, derived by a variety of procedures from almost every animal tissue, and universally described as "hormones". Bayliss and Starling (1902) had intended this word to apply to an active principle formed in one organ and carried in the blood stream to another organ or organs where it produced its specific effect. Their discovery of "secretin" had certainly required such a definition but its subsequent usage had not been respected. This situation was not clarified until 1929 when Dale (1929) and Trendelenburg (1929) carefully explained the word and gave it the significance it holds today. Thus "hormone" is defined "a chemical substance formed in one organ or part of the body and carried in the blood to another organ or part which it stimulates to functional activity". Adrenin, secretin and thyroxin are typical examples of such a substance. In contrast, other pharmacologically active substances seem to be formed unspecifically by almost any organ, tissue or tissue juice. Feldberg and Schilf (1930) have given the name "Gewebshormone"

or "Gewebstoffe" to these substances. Their normal metabolic action was believed by Dale (1920) and Lewis (1927) to be of a local nature.

However, such "tissue hormones" as histamine and acetylcholine are also capable of acting, as will be shown, on organs some distance from their site of production. As far as can be determined only 5 tissue hormones have been given a definite chemical composition. These are histamine, acetylcholine, carbon dioxide, adenylic acid and serotonin (5-hydroxy tryptamine).

In the same class, possibly, are such less specific substances as kallikrein (Frey and Kraut (1928);Substance P, (v.Euler and Gaddum,1931); "slow-reacting-substance"(Feldberg and Kellaway,1938; Brockelhurst,1953), and Bradykinin(Rocha e Silva, Beraldo, and Rosenfeld 1949). The nonspecific vascular action of these unidentifiable pressor or depressor substances has usually caused them to be classified together.

When Vincent and Sheen (1903) noted the presence of a depressor substance in tissue extracts, the early phase of histamine research began. Popielski (1909) gave the name "vasodilatin" to a similar extract, but this was merely a hypothetical designation for an unknown substance. The synthesis of histamine in 1907 (Windaus and Vogt) was soon followed by its isolation from ergot (Barger and Dale 1910) and its characterization (Dale and Laidlaw 1910). Later experiments, mainly by Dale, provided evidence for the possibility that such substances as vasodilatin may actually be histamine or at least contain it.

Biedl and Kraus (1910) had already drawn attention to the close similarity between the symptoms of anaphylactic and peptone shock when, a few months later, Dale and Laidlaw (1910) found that histamine behaved almost identically to peptone. Anxious to prove their suspicion that histamine was a common factor in all these conditions, Barger and Dale (1911) isolated histamine from intestinal mucosa and thus had evidence that

that depressor tissue extracts could contain histamine. Their choice of tissue, however, proved to be an unfortunate one because Ackermann (1910) had already shown that histamine could be prepared by the action of putrefactive microorganisms on histidine. When Mellanby and Twort (1912) reported the isolation of an intestinal bacillus capable of making the same conversion, the probability that histamine was a natural constituent of the intestinal mucosa could not be accepted.

The heretofore mentioned phenomenon of anaphylaxis was actually first described in 1902 by Portier and Richet. They found that when a dog, who had recovered from one dose of an extract of sea actinia, received a second and smaller dose several weeks later, it exhibited an extreme picture of intoxication and shock. A similar loss of natural protection was described in rabbits by Arthus (1903) and in guinea pigs by Otto in (1906).

During the next 20 odd years a good deal of controversy raged over histamine's part in both anaphylactic and peptone shock, but in the absence of conclusive evidence little more than theories could be proposed. However, in the search for answers to these problems a wealth of knowledge was accumulated and the characterization of these reactions became almost complete.

In 1920 Dale postulated that in anaphylactic shock an inter-reaction between antigen and antibody caused a cellular injury which then liberated histamine and other substances. Similarly peptone was believed to involve cell injury when it produced its effect. In 1929, Dale reaffirmed his views but this time largely relied on evidence produced by Sir Thomas Lewis,(1927). Lewis had found in a series of classical studies on the small blood vessels of the human skin that a large variety of mildly injurious stimuli resulted in the production of a common reaction which he termed the triple response. Lewis believed that this reaction was caused by the activation of a chemical

agent and when, at Dale's suggestion, he tested histamine and found that it reproduced in detail the triple response his suspicions were all but confirmed. However, he permitted no closer identification of the natural agent in the tissue reaction than to call it "H-substance". It is notable that Dale's suspicions of 20 years before had not yet been verified.

Best and McHenry (1931) attribute the slow progress made to the lack of a sensitive method for the estimation of histamine in blood. Whatever the cause, the introduction of such a method by Barsoum and Gaddum (1935a) ended the period of doubt, and it was not very long before adequate proof for the presence of histamine in tissues was obtained. Subsequent improvements of their method, mainly by Code, (1937) have produced a technique still very much in use.

A fresh impetus was gained in histamine research when MacIntosh and Paton (1949) described a number of experiments characterizing histamine release. For the first timesubstances were classified as "histamine liberators" and were identified as probably having a specific chemical structure. Since then many compounds possessing the proposed structure have been shown to fit into the pattern as "releasers" or "liberators" even though their more widely recognized pharmacological effects are of an entirely different nature.

In my laboratory a number of details regarding the mode of action of histamine liberators have been investigated and are herein described. In particular, as a means of approach to the general problem methods attempted for modification of histamine release are discussed.

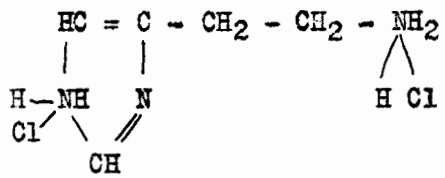
The preceding outline has briefly sketched the history of histamine research from the time of its vague beginnings to a more definite phase in which has been introduced the study of histamine release. There now follows

a review of the literature on histamine with sections devoted to its pharmacological action; substances, techniques, and reactions which bring about the release of histamine; and various theories which attempt to explain the manner by which histamine is liberated. No attempt has been made on the part of the author to review the great mass of literature on histamine, but those details of pertinent investigations concerning the present study have been carefully considered. Even in the respect of histamine release the number of studies mentioned represents only a portion of the investigations on the subject. A more complete list of histamine releasing substances together with their references appears in the Appendix.

The pharmacological actions and other properties of histamine have been described in a number of excellent monographs and reviews. (Feldberg and Schilf 1930; Best and McHenry 1931; Gaddum and Dale 1936; Rose 1939; Dragstedt 1941) Because of this, the author presents here only those details of the earlier manuscripts which are applicable to the investigations undertaken.

B. Chemistry and Distribution of Histamine

Histamine is β-iminazolylethylamine, the decarboxylated product of the amine acid histidine. The molecule is basic by virtue of its amine groups which permits it combination with acids to form stable salts. The molecular weight of the base is 111. The dihydrochloride (molecular weight = 183) and the diphosphate (molecular weight = 307) are the two most commonly used salts and have respectively 60% and 30% histamine. The formula of the dihydrochloride is as follows:



Because of the acidity of the salt, strong solutions of histamine for use in pharmacological studies should be made up in buffered media.

In the body histamine is detoxified by an enzyme system termed "histaminase" by Best and McHenry (1930). Histaminase was later identified by Zeller (1938) to be a diamine oxidaze of the flavoprotein type and Kappeler-Adler (1950) has found evidence to suggest that the prosthetic group of histaminase is flavin adenine dinucleotide.

Although histaminase is widely distributed throughout the animal body, the kidney contains the greatest concentration (McHenry and Gavin 1932). During pregnancy the decidual tissues show a marked degree of histaminase activity (Roberts and Robson 1952). Its presence in bacteria (Roulet and Zeller 1945) suggests the reason why histamine solutions should only be kept as sterile solutions in an ice box. Pure solutions of histamine may be boiled in acid for long periods without loss of potency.

Best and McHenry (1931) have discussed in some detail the wide distribution of histamine in both plant and animal tissues. Barger and Dale (1910) isolated histamine from ergot and the subsequent studies of Dale and others showed that histamine possessed important pharmacological actions. The isolation of histamine from other plants has had little bearing on the elucidation of its pharmacological role.

Among animal tissues that contain histamine the highest concentrations are found in the liver, lungs, and skin. The values depend to a great extent on the species but there is also considerable variation in the same organs of different individuals of the same species (Feldberg and Schilf 1930). Histamine is normally combined in the tissues in a form which prevents its acting locally or being released in the circulation. Although it is believed to have a local action, its regular presence in the blood stream of most

species indicates the possibility of a more generalized function in the body. The values of histamine in the circulation range from 0-0.04 µg./ml. in the dog to 1-4 µg./ml in the rabbit with intermediate values found in the cat, guinea pig and man. (Code 1952). In his review, Code (1952) concludes that the main source of blood histamine is the granular series of leucocytes although these may not always be "loaded". He also notes that the free plasma histamine may come from other body tissues.

C. The Pharmacology of Histamine

The significance of the presence of histamine in the body is not clearly understood but functions most commonly ascribed to it are;

(a) Vasodilatation of the small vessels of the circulatory system.

(b) Stimulation of gastric acid secretion.

(c) Transmission of synaptic impulses in the central nervous system

The participation of histamine in the placentation of the ovum has been suggested by some very recent investigations by Shelesnyak (1952).

The early concept of the possibility that histamine played an active part in the control of capillary circulation was thoroughly discussed by Dale in the first Herter Lecture (1920). Although it had not been shown that any specific metabolite was involved in the mechanism Dale was of the opinion that "control could best be affected by the local action of metabolic products of activity". Metabolic agents that have been identified as taking part in the capillary control of local blood flow are members of the adenosine group, carbon dioxide and sometimes, histamine. Capillary "vasomotion" is believed to play a major role in the regulation of capillary permeability and peripheral blood flow (Chambers and Zweifach 1947; and Chambers 1948). It was suggested that the degree of this activity in small vessels was under the control of local metabolic products.

The following is a summary of the more important effects of histamine. Longer detailed accounts are found in the monographs and reviews by Feldberg and Schilf (1930) Best and McHenry (1931) Rose (1939) Selle (1946) and in the conference on histamine (Ann. N.Y. Acad Sci 1950).

1. The Effect of Histamine on the Vascular System

(a) General Circulation

The injection of as little as 0.1 µg histamine into any mammal is usually followed by a small transient fall in arterial pressure. This effect is due to a relaxation of the minute arterioles and capillaries. Larger vessels usually undergo a constricting action (Dale and Richards 1918; 1927; Burn and Dale 1926). The level at which the changeover occurs varies with the species. In the dog and rabbit visible arterioles, as well as the capillaries, relax to histamine but in the cat only the finest vessels relax. Generally, the vasodilator effect predominates but this depends on such factors as oxygen and adrenalin necessary for the maintenance of capillary tone. If the tone is lost vasodilation becomes impossible and the only effect seen is a blood pressure rise due to larger vessel vasoconstriction. Recent work on very small vessels suggests there may be several kinds of locus where histamine may act differently; metarterioles, thoroughfare channels, A.V. anastomoses, precapillary sphincters. (Chambers and Zweifach 1944).

(b) Heart

Small doses of histamine increase the heart's rate and strength of contraction. Larger doses weaken the heart, the left side being more affected. Klisiecki and Holdbut (1937) state that the action of histamine on the heart may be the primary cause of histamine shock in most animals.

(c) Portal Circulation

In 1932 Bauer, Dale, Poulsson and Richards found that the smaller hepatic arteries of the dog dilated to histamine while the portal branches

were slightly constricted. In the dog a valve-like contraction of the hepatic veins led to a profound engourgement of the liver. (Mautner and Pick 1929). Dale and Laidlaw (1911) found that this contraction was followed by a greatly increased flow of liver lymph. It will be seen that these signs are also associated with conditions of induced histamine release.

2. The Action of Histamine on Smooth Muscle

Throughout the body smooth muscle is contracted by histamine. The muscled portions of the uterus, intestine and bronchioles are most sensitive to histamine somewhat depending on the species. Because of their high sensitivity to histamine, the uterus and intestine of the guinea pig have been widely used for assay purposes. Dale and Laidlaw (1910) also used the retractor penis muscle of the dog as a test object for histamine.

(a) Uterus

Dale and Laidlaw (1910) showed that the virgin guinea pig uterus would respond to histamine in concentrations as low as 1/250,000,000. The concentrations necessary for contraction in other species was found to be much higher. The isolated rat uterus responds to histamine by a depression in its natural rhythm. (Suden 1934). Although the sensitivity of the uterus is reduced in pregnancy, some uteri are more sensative to histamine at term. (Bourne and Burn 1927 Dale and Laidlaw 1910).

(b) Intestine

In vivo, large doses of histamine may cause purging and emesis (Dale & Laidlaw 1910). The isolated suspended gut is especially sensitive to histamine, that of the guinea pig responding by contraction to concentrations as low as 1/250,000,000. Since the guinea pig preparation also responds in a graded manner to the concentration of histamine in the surrounding bath (Guggenhein and Leeffler (1916) it can be used for the determination of histamine.

Barsoum and Gaddum (1935) have used the rectal caecum preparation of the chick for the estimation of histamine.

(c) Bronchioles

In most animals the pulmonary vessels are constricted by histamine. (Dale and Laidlaw 1910). Isolated rings of these vessels also constrict in the presence of histamine. (Feldberg and Schilf 1930). This action in whole animals causes an interference with the right ventricular output, and, especially in rabbits, right heart failure may cause death. In the guinea pig the picture of shock is dominated by an asphyxiating constriction of the bronchioles and the animal dies of bronchospasm. Schild, Mongar, Hawkins and Herxheimer (1951) have demonstrated the action of histamine and specific antigens on human bronchial muscle and noted that it is quite as sensitive as the guinea pig preparation.

(3) Action of Histamine on the Skin

The interesting effects of histamine on the human skin were first noticed by Eppinger in 1913, but the studies of Lewis (1927) completely overshadow any work before or since and are very readable today. The reaction of an amount of histamine which can enter the skin through the smallest pin prick is essentially the same as that caused by a minute mechanical or chemical injury. Three separate effects which usually appear together after histamine enters the skin have been called the "triple response". These are as follows:

1. A local reddening appears at the site of injection beginning in about 15 seconds and is due to a vasodilatation of the small local vessels contacted by histamine. This action is not affected by degeneration of the local nerves and is solely dependant upon the vasodilatation of the capillaries.

2. A diffuse flare surrounds the local redness after another 10-30 seconds. This "scarlet flush" relies on a local axon reflex to local arterioles and cannot be obtained after nervous degeneration in the area. Loesser (1938) has made use of this phenomenon for determination of an intact nerve supply in patients.

3. A wheal which appears within a period of 3 to 5 minutes covers the same area previously showing the local reddening. This is caused by an increased permeability of the fully dilated capillaries in the area and may last an hour or more.

4. Lethal Doses of Histamine by Intravenous Injection

The following values in milligrams/Kg. of body weight are the approximate lethal doses by intravenous injection (Best and McHenry 1931):

Guinea pig	.250 - .30	Pigeon	1-2	Cat		Rat	300
Rabbit	1-3	Dog	2.5-3	Mouse	250	Frog	1700

5. Agents that Counteract the Action of Histamine

The apparently harmful presence of histamine in allergic and other conditions has stimulated the search for methods for histamine counteraction. These studies have had to take into account that normal functions of histamine in the body would not be impaired by the application of these methods and that the detoxifying mechanism would not elicit unwanted side effects. The development of these methods has of course, been beneficial to the advancement of other investigations on histamine. Although several methods for detoxifying histamine or counteracting its effects have been attempted, few have had the usefulness and widespread application of the modern anthistaminic agents. Earlier, histamine azoproteins had been investigated for their value in allergic phenomena (Fell 1950; Coffin, Kabat 1946) but had only a limited application. Negative results with such substances have indicated a poor antigenicity.

Desensitization of animals with large doses of histamine(Alexander 1944: Rose and Brown 1948) has not had much value in humans as a practical method for counteracting the effects of histamine. Karady (1941) has presented the most convincing evidence that pretreatment with histamine is a useful procedure for desensitizing to histamine. However his experiments were done on guinea pigs subjected to anaphylactic shock - a phenomenon in which other factors besides histamine may be involved. Histaminase has been used for the same purpose but without consistent success. (Alexander and Bottom 1940; Rose and Browne 1941).

Trethewie (1951) found that sodium salicylate and acetyl salicylic acid inhibited the release of histamine from sensitized lungs. Gray, Pedrick and Winne (1951) have confirmed these results for sodium salicylate. Gray also found that ethyl alcohol could abolish the anaphylactic response of the guinea pig ileum to antigen but the mechanism for this action, as with the others remains obscure. The continued use of salicylates in allergic diseases of many kinds has shown that these substances have a valuable place in modern therapy. The claim by immunologists that rheumatism and related ailments are allergic phenomena is supported by the knowledge that they can be successfully treated with salicylates.

Ungar, Parrot and Bovet (1937) found that the sypathicomimetic agents adrenalin and ephedrine possessed antihistamine activity. Adrenalin was observed to be effective in concentrations as low as 10^{-8}. The sympathicolytic agent yohimbine was also shown to possess antihistamine activity.

The discovery of Thymoxyethyldiethylamine (929 F*)by Bovet (see 1950) and characterization by Staub has led to today's highly specific compounds of practical usefulness. The effects of 929 F were threefold (Bovet and Staub 1937; Staub 1939).

(a) Protection to guinea pigs against several lethal doses of histamine.

*Fourneau

(b) Antagonism against histamine-induced spasms of intestine, uterus and bronchi.

(c) Protection against experimental anaphylactic shock.

In 1942, Antergan was prepared by Mosnier and investigated by Halpern (Halpern 1942). Its antihistamine action was found to be several times greater than that of 929 F. Later, Neoantergan was developed and was found to have an anti-histamine activity 60 times greater than that of 929 F. Although other compounds have been found to have greater anti-histaminic effects, their toxicities are proportionately very much greater. The therapeutic index of Neoantergan is 68,000.

With the advent of these new substances the specific actions of histamine could be measured with extreme accuracy and while the field seemed to have enlarged to immense proportions in a moment it had also become simplified. Staub (1939) was the first to show the protection in guinea pigs to histaminic bronchoconstriction. Practically every known effect of histamine was later shown to be abolished with one or other of the new substances. In North America, Benadryl was the early champion of antihistaminic compounds (Loew and Kaiser 1945; McElin and Horton 1945).

The dose of Neoantergan that inhibits histamine has frequently been found to be smaller than the dose of histamine that produces contractions of suspended gut preparations (Schild 1947). Personal observations (Ashwin 1951) have shown that the inhibiting action of Neoantergan on the effect of histamine in the same preparation is up to 100 times more specific than the action of atropine against acetylcholine. 100000 times the dose of Neoantergan used to inhibit a constant histamine response was necessary to abolish the constant action of acetylcholine whereas only 800 times the amount of atropine used to abolish the response to acetylcholine was required to prevent the action of histamine.

Rosenthal and Brown (1940) have shown that 929 F annuls anaphylaxis in guinea pigs and others have demonstrated the effectiveness of these compounds

in the anaphylactic conditions of rabbits (Vallery-Radot, Bovet, Mauric and Holtzler 1943) and dogs (Halpern 1942).

Herxheimer (1952) and also Armitage, Herxheimer and Rosa(1952) found that mild anaphylactic shocks in guinea pigs produced by aerosols containing the antigen could be protected by a number of antihistamines. The protective effect consisted in delaying the onset of the asthmatic dyspnoea due to the shock. Few animals were completely protected by the antihistamines. Atropine and procaine had similar but weaker anti-anaphylactic effects. The authors discuss the parallel of this protection to that exhibited by antihistamines against human asthma.

Schild and co-workers (1951) have shown that the antihistamines are not very active against allergic reactions involving the plain muscle of the human bronchi preparation. The discrepancy was thought to be due to the large concentrations of locally released histamine where there was an insufficient amount of antihistamine. The manifest action of antihistamines in the cellular mechanism involving anaphylaxis and the subsequent release of histamine was certainly much less than its inhibitory effect against added histamine.

Antihistamines have been shown to exert little, if any, inhibitory action against anaphylaxis in dogs (Wells, Morris, Dragstedt 1946). Apparently histamine is still released in the presence of the antihistamine but the animal's protection against the released toxins has been greatly increased. Paton and Schachter (1951) have come to the same conclusion for the histamine liberator 48/80. After an injection of 48/80 into an unanesthetized dog, signs associated with histamine release were diminished considerably with mepyramine (Neoantergan) but the amount of gastric secretion, which they used as the criterion of histamine released, was not affected.

In mice antihistamines have been found ineffective against both histamine intoxication and anaphylaxis. (Mayer 1950). Mayer has assumed that there are probably other anaphylatoxins in addition to histamine. While in other species, such as the guinea pig, the effects of allergic responses are easily annulled by antihistamines it is not permissible to say that other hidden manifestations of the primary effect are not also inhibited. Mayer suggests that the drugs, specific as they seem to be against histamine, probably have other actions as well in producing relief and these secondary effects may be intrinsically linked to antihistaminic activity. Such side effects have been listed by Feinberg (1947):

1. Atropine-like activity on smooth muscle and glands.
2. Local anesthetic activity.
3. Excitatory to the central nervous system.
4. Recently another possible reason for the ineffectiveness of antihistamines has come to light. Pellerat and Murat (1946) and Arunlakshana (1952) have found that various antihistamines themselves release histamine. However this effect does not occur until comparatively large amounts of the antihistamine are given to the animal. Even though antihistamines apparently do not prevent the release of histamine from tissues, they probably do prevent many of the actions of the released histamine on the organism.

D. The Liberation of Histamine

This section deals with a number of reactions, events and substances which cause the liberation of histamine.

Although the extraction of histamine from tissues is a relatively simple procedure, the identification of histamine released from the same tissues when they are subjected to a certain stress or procedure has been at times exceedingly difficult. The failure of many attempts to detect the presence of histamine pharmacologically in blood, plasma or perfusates has been attributed to the presence in them of other active substances which obscured the action of the histamine. When Best and McHenry (1930) introduced a method for the determination of tissue histamine employing acid hydrolysis many of these difficulties were removed, and when Barsoum and Gaddum (1935) published their method for the estimation of histamine in blood, further investigations in this line could be pursued with added confidence. However, some authors still refer to the active ingredient in blood and tissue extracts as "histamine-like"substance or "H" substance.

Usually an increased level of histamine in the blood or perfusion fluid has been taken as the criterion for its release from the whole animal or from a specific area or organ and yet, when blood was used, a concomitant release of histamine from red and white corpuscles was sometimes a matter of uncertainty. Code (1952) has emphasized that histamine is easily released from blood cells during clotting and also in the presence of some anti-coagulants.

In some reports, methods for the identification of histamine have been faulty or inadequate and the results cannot be accepted. In others, if the histamine level was not over a certain high percentage a claim for release could not be made.

The advent of antihistaminics provided methods for the irrefutable identification of histamine. The action of these new compounds was so specific that other methods were almost unnecessary. Many investigators today do not even attempt absolute purification of their samples because the antihistamines and atropine are available to ascertain the presence of other substances which may interfere in the pharmacological assay of histamine. Measurement of other factors, such as the "slow reacting substance" (Feldberg and Kellaway 1938: Brocklehurst 1953) is also possible by these new techniques and their release together with histamine in many phenomena is demonstrable.

Along with the growth of proof that the dynamic factor in anaphylactic and anaphylactoid conditions was histamine, the knowledge of how it was released also grew. Although the presence of histamine in both types of phenomena was fairly well accepted one was unable to deny that there were many differences. Today's familiar symptoms are often attributed to the agent used rather than to histamine released. Other less well identified components in the reaction are also under suspicion of having diverse effects on the animal.

1. The Release of Histamine During Metabolic Activity

One of the most perplexing problems in the recent history of physiology has been whether histamine has a normal metabolic function in the animal's body. The presence of so potent a molecule as histamine in the tissues would certainly seem to be due to a more useful function than to merely represent, as a waste product, the essential amino acid histidine. The release of histamine, whether described as a "normal" or a "pathological" process, has not been shown to be due to either the acceleration of a normal process or to the onset of a mechanism not usually occurring in the metabolism of the cell.

A few of the mechanisms suggested to mediate the release of histamine are:

(a) The decarboxylation of histidine

(b) The splitting of peptide or other bonds after the activation of an enzyme

(c) The disruption of physical forces holding histamine in the cell

(a) The Release of Histamine in the Regulation of Local Blood Flow

As early as 1920 Dale mentioned that the control of local circulation could probably be best affected by the metabolic products of local activity. At the time the presence of such agents in tissues was known but whether they had a definite role in living tissue had not yet been demonstrated.

Probably the experiments by Lewis (1927) were the first to suggest that a "histamine-like-substance" was released during such conditions as reactive hyperaemia, oxygen-want, and carbon dioxide increase. By using an improved method for histamine extraction,Barsoum and Gaddum (1935B) were able to demonstrate, that this probably did take place. The results of their experiments with CO_2 suggested that the release of histamine was a secondary consequence to the accumulation of acid metabolites. During reactive hyperaemia they found that the histamine concentration of the blood was increased several times over the normal level.

Anrep and Barsoum (1935) showed that the amount of histamine in the venous blood of a dog's gastrocnemius muscle increased inversely as the rate of perfusion. Anrep et al (1944) later showed that muscular contraction, arterial occlusion, and prolonged venous congestion in the human subject were all accompanied by a release of tissue histamine. Their conclusions were in accordance with the observations of Lewis (1927) who had found evidence to suggest that histamine was much more conspicuous in venous blood during muscular contractions.

Tarras-Wahlberg (1936) was able to show in rabbits that the venous blood but not the arterial blood, possessed histamine-like activity. This activity was further increased by asphyxia or haemorrhage.

Eichler and Speda (1938) found evidence to suggest that oxygen-want may be a factor in the normal release of tissue histamine. After giving cats artificial hyperventilation the plasma histamine was found to be at a very low level. This returned to a normal value only after a period of several hours. Hyperventilation induced by hyperthermia or cardiazol produced similar values. When the conditions were reversed by giving cats 10% oxygen to breathe, the plasma histamine was increased to extremely high levels. (Eichler, Speda and Wolff 1943).

Feldberg and Holmes (1941) have been able to increase the amount of gastric acid secretion presumed by them to be due to histamine in the circulation, by decerebrating cats. During the (decerebrate) rigidity there was an increased amount of acid formed. Similarly, intense rigor in leg muscles, caused by strong faradic impulses, was followed by an increased production of gastric acid. Throughout the rigor, secretion remained at a high level and only passed off with cessation of the contraction or amputation of the limb.

Using heart lung preparations, the release of histamine during increased cardiac activity has been demonstrated by Anrep, Barsoum and Talaat (1936) and by Marco and Parhon (1938). In both cases the coronary venous blood obtained from vigorously contracting hearts could be shown to contain increased amounts of histamine. Anrep et al (1939) also showed that there was a release of histamine following the administration of adrenalin to an isolated rabbit heart. This could not be shown for a similar cat preparation. Code, Lovatt-Evans, and Gregory (1938) could not confirm the increased histamine level in coronary venous blood and also criticized the earlier demonstrations on the basis that the methods then used were not sufficiently accurate to produce reliable results. On the basis of the careful work of Code and his associates in which a very delicate method for blood histamine

was employed, some doubt has been cast on the reliability of the experimental values found by Anrep and his coworkers.

These somewhat doubtful experimental findings have presented evidence that the liberation of histamine is a normal cellular activity adequately accounting for functional vasodilatation. "If this functional liberation is to be accepted", states Dale,"the question still remains whether it (histamine) acts by itself or by a synergism with CO_2, a possibility suggested by observations recently shown to me by H. Rein in Gottingen". It is notable that Dr. Dale's observation had suggested to him a mechanism for histamine release similar to that suggested to Barsoum and Gaddum(1935B) 15 years before.

(b) The Release of Histamine and Placentation of the Ovum

Another body process that employs the mechanism of histamine release is suggested by the investigations of Shelesnyak (1952). His studies suggest that natural placentation of the rat ovum is initiated by the release of histamine. The formation of artificially produced deciduoma was inhibited if the animals were treated with antihistaminic compounds. Unpublished data of Shelesnyak and Davies (See Shelesnyak 1952) suggested to them that in the mouse treated with benadryl during the time of natural placentation interference with nidation occurred since pregnancy did not go to term.

If such mechanism as circulatory adjustment and natural placentation are actually mediated by the release of histamine, the use of antihistamines to give release from various distressing symptoms, due to pathologically liberated histamine, must be cautioned against so that normal and essential physiological adjustments are not harmfully suppressed.

(c) The Release of Histamine by Nerve Stimulation

Because the release of histamine by nervous mechanisms has little bearing on the present study only a few details are mentioned.

By using acid gastric secretion as the criterion of histamine activity

Ungar (1937) and Ungar and Zerling (1935) have found that various types of nervous stimulation liberated a "histamine-like-substance".

MacIntosh (1938) believes that histamine may mediate the secretory action of the vagus on the parietal cells and therefore act as the normal stimulant to gastric secretion. However when blood was withdrawn from dogs and tested for the presence of histamine while the dogs were digesting a meal no histamine could be detected.

Such substances as acetyl choline(Dale and Gaddum 1930; and kallikrein Ungar and Parrot 1936) are also released on nervous stimulation. Whether these play any part in the gastric secretory mechanism is not known.

Good evidence that antidromic stimulation of sensory nerves released histamine has been produced by Kwiatkowski (1943) who demonstrated the presence of histamine in the venous blood after the stimulation of cut dorsal roots. Lambert and Rosenthal (1943) have described the liberation of histamine from the appropriate skin area following cervical sympathetic or splanchnic stimulation. Such studies suggested the presence of the so-called "histaminergic" nerves. The local anesthetic action of the anti-histaminics now in use offers indirect support for this hypothesis.

2. THE RELEASE OF HISTAMINE IN ANAPHYLAXIS

Richet (1902) was the first to make an analysis of the reaction to which he had given the name "anaphylaxis". He and other investigators observed that some anaphylactic phenomena had a similarity to immunological processes. The statements of Dale (1920) help to clarify this relationship.

> "Anaphylaxis is not, as its' name was intended to imply, the opposite or antithesis of immunity but a phase in the development of immunity in which the immune substance or anti-body has a peculiar distribution."---" Fundamentally, the immune

reaction of which anaphylaxis is a specialized phase, represents the need of the species to prevent the permanent incorporation into their cells of protein having a different type from its own."

Extending Richet's observations, Biedl and Kraus in 1909, and Arthus in 1910, reported that the fall of anterial pressure during anaphylaxis in laboratory animals occurred subsequent to the vascular reaction which they regarded as the most important event. They believed the fall to be due to a vasomotor paralysis not connected to the action of the heart, and suggested because of the striking resemblance of anaphylaxis to the shock caused by peptone, that anaphylaxis was probably a peptone intoxication. When Dale and Laidlaw (1910) compared these two conditions to those observed during histamine shock they suspected the former to be primarily the result of a release of histamine.

Because of the great number of papers written on anaphylactic reactions in the mammalian species only those portions which are applicable to the present investigations have been herein discussed. In particular, the steps leading to the release of histamine in anaphylaxis have been described in detail with emphasis placed on the systems which eventually cause the release of histamine from the body's tissues. Together with the consideration of species differences, the significance of histamine in accounting for the symptoms of anaphylaxis has been assessed.

The release of histamine in anaphylactic shock will now be described as it occurs in various species. In most cases, unless otherwise specified, the antigen has been either egg-white or horse serum. It should be noted that a number of chemical substances are available to be used as sensitizing agents in addition to various proteins. Arsenobenzene, for example, has

been used by Simon and Staub, (1937) and many of the sulpha-compounds are also potent antigens. (Boyd, 1947) These chemicals acquire their antigenic properties indirectly through binding with body proteins.

(a) Histamine Release in Canine Anaphylaxis

When an unanesthetized dog, sensitized to a foreign protein receives a week or so later, a second injection of the same protein, the dog becomes in most cases, acutely sick. The symptoms observed are salivation, scratching, vomiting, defecation, etc. The animal may then collapse and die, or, after a period of coma, return to normal. Some animals have only a mild reaction. Those animals that recover after a severe "shock" are usually refractory to subsequent doses of the antigen.

Under ether anesthesia, the anaphylactic dog exhibits a rapid fall in arterial pressure and this has been most often attributed to an increased level of blood histamine. Lengthened coagulation times during the period of anaphylaxis have been shown to be due to the presence of heparin in the blood stream. Both heparin and histamine apparently enter the circulation from the liver; the organ primarily involved in the reaction. The livers of such anaphylactic dogs are engorged with blood and on gross observation appear very dark. Canine anaphylactic blood is markedly cyanotic for want of oxygen, and has a high hematocrit because of plasma leakage from the capillaries. The red cell count is relatively normal but the white cells and platelets are reduced in number.

These follows a more detailed account of these conditions as they are found in canine anaphylactic shock. The description is important because of the similarity of these conditions with those in which the reactions have been initiated by "histamine liberators".

The earliest work by Manwaring on dogs (1910) indicated that an organ

located below the diaphragm was responsible for anaphylactic shock. This was shown by a simple ligation of the aorta and the inferior vena cava after which treatment, when the antigen was given, no shock occurred. Two of his experiments indicated that the liver was responsible for releasing the blood-borne toxic agent which caused the shock. Firstly, dogs with liver shunts did not manifest anaphylaxis. Secondly, in cross-circulation experiments between normal and sensitized dogs he found a fall in arterial pressure in the normal animal-supposedly due to the blood borne toxin. Voegtlin and Berheim (1911) were able to confirm Manwaring's first observation using dogs with Eck fistulas. However, Weil (1917) and Weil and Eggleston (1917) claimed that in the second experiment the normal animal's arterial pressure would fall by the drainage of blood into the anaphylactic animal. Weil also found that no toxin could be liberated by perfusing normal or sensitized livers with blood containing antigen.

Manwaring (1921) produced evidence that a vasodilator substance was produced in the dogs liver during the anaphylactic reaction but this (or hypotension) did not occur when dogs were hepatectomized. Comparing the action of histamine in large doses to that manifested in anaphylactic and peptone shocks, Manwaring found that hepatectomy reduced the severity of the peptone reaction. He felt that the three conditions: histamine, anaphylactic- and anaphylactoid shock so alike in symptoms were probably not initially or fundamentally identical physiological mechanisms.

By using the lower quarters of a normal dog and the top half of an anaphylactic animal connected only by circulation Manwaring and associates (1925) produced more evidence that a smooth muscle stimulating substance, not unlike histamine, circulated in the shocked animal. However their only criterion was the appearance of contractions of the bladder and intestine. A toxin had certainly been elaborated but its nature was still in doubt.

A marked increase in the flow of lymph from the thoracic duct in anaphylaxis or with obstruction of the hepatic veins was noticed by a number of workers (Calvary 1909) (Peterson and Levinson 1923; Simonds and Brandes 1927).

Dragstedt and Gebauer Fuelnegg (1932a) were prompted by these observations to look for an anaphylatoxin in the thoracic lymph duct. A smooth muscle stimulating agent was found to appear rapidly as the reaction developed. They (Dragstedt and Gebauer Fuelnegg 1932 B) were able to show that the substance exhibited properties very similar to those of histamine. It was a dialyzable crystalloid of basic properties, stable to boiling with acid, contracted the guinea pig gut but not that of the mouse, lowered the cats arterial pressure and produced histamine-like wheals in human skin . In 1936 Dragstedt and Mead showed that the enzyme histaminase was able to inactivate the substance responsible for these effects.

While some workers were attempting to find and identify substances responsible for the symptoms of anaphylaxis, others focussed their attention on the liver as the active site of the reaction. In 1925 Manwaring had shown the sensitivity of a dog to antigen was almost limited to the liver cells (Manwaring, et al, 1925). Watanabe (1931) reported the apparent loss of a high percentage of liver histamine during anaphylaxis but his values represented group results and did not reflect measured changes in any one animal. It is now generally accepted that in the dog the major anaphylactic reactions are associated with the liver even though some manifestations undoubtedly occur in the absence of this organ (Davidoff, Kopellof, 1931).

Dragstedt and Mead (1936) tested blood samples for histamine in a large number of dogs in anaphylactic shock and were able to ascertain how much histamine was elaborated during the reaction subsequent to its iniation. By comparing the rate at which injected histamine disap-

peared from the blood they concluded that the vascular reaction in anaplylaxis could be completely accounted for by the amount of histamine thus computed. Code (1939) confirmed this evidence using an improved method of histamine purification.

Code & Ing(1937) were able to isolate crystalline histamine from anaphylactic blood. Code (1939) found that dog blood histamine increased almost one hundred times, very quickly after the antigen was administered reaching a value of 1.0 ug/ml. The disappearance of this histamine differed from that of injected histamine in that it remained at a supranormal level for 2 or more hours. This suggested a short "explosive" release followed by a long lasting slow output.

In 1924, Webb was able to demonstrate histologically an enormous accumulation of leucocytes within the liver parenchyme of the dog during the leucopenia of anaphylaxis. Later Kinsell and associates (1941) formed a theory correlating the disintegration of platelets and leucocytes with the liberation of histamine. Their experiments, however were done on rabbits and it is not so easy to apply this theory to explain liberation of histamine in the dog where platelets are relatively scarce. Much earlier, Wolf (1908 and 1910) had shown that in both anaphylactic and peptone shock, a proteolytic enzyme is activated. But whether the engourgement of the liver with white cells and its release of histamine by proteolysis could be reconciled in one scheme was barely attempted until very recently.

Rocha e Silva and co-workers (1951) have correlated many of the conditions in which histamine is released by postulating the presence in circulating blood of a powerful histamine releasing agent which is easily activated in these phenomena. Their experiments (Rocha e Silva 1950) have ascribed to platelets and white cells a role of paramount importance.

It had been known that often an antigen did not cause a fall in blood pressure (Fidlar and Waters 1945) but there was a consistent reduction in circulating platelets and white blood cells. With peptone the constant shock was also accompanied by a fall in these elements. Ojers et al (1941) have also suggested that a blood constituent is necessary for a maximum release of histamine in anaphylactic shock in the dog.

Rocha e Silva (1950) found that anaphylactic shock in the dog was paralleled by a massive disintegration of the cells and platelets clumped together in the liver. The presence of a responsible fibrinolytic enzyme within them was indicated and subsequent experiments provided sufficient proof of this. The manner by which the antibody-antigen reaction sets off the clumping-disintegration mechanism of the leucocytes is not yet known.

Concurrently with histamine, the release of heparin from the liver of dogs during anaphylactic shock is now a well known fact. The striking loss of coagulability of the blood during anaphylactic and peptone shock was noted by many of the early workers notably Biedl and Kraus, and Arthus. This loss of clotting power was attributed to a variety of factors but Waters, Markowitz and Jaques (1938) were the first to demontrate an increased titre of heparin in the blood of such animals. Jaques and Waters (1940) completed proof by isolating crystalline heparin from anaphylactic blood. Best and co-workers (Best, Cowan, Maclean, 1938) have noted that while the release of heparin is very intimately associated with the release of histamine in these conditions, there is no reason to suppose that it contributes to any of the vascular or other symptoms.

The regular occurence of both histamine and heparin in these conditions has led to the theory of a common cellular origin. A recent investigation

by Riley and West (1952) has shown a high correlation in many tissues, between the number of mast cells and the measurable amount of histamine and heparin.

(b) The Release of Histamine in Guinea Pig Anaphylaxis:

Auer and Lewis (1909; 1910) were the first to analyse the physiological machanism involved in anaphylactic shock in the guinea pig. They demonstrated that the respiratory difficulty and asphyxia were due to a swiftly developing stenosis of the bronchioles which prevented the exchange of air even during violent respiratory efforts. Dale (1912-13) later demonstrated that this bronchospasm could be reproduced in the isolated perfused lung and thus concluded that nervous influences did not take part in the reaction.

With the guinea pig anaphylactic condition clearly defined as involving the contraction of smooth muscles alone, it was natural to look for the toxins responsible for its genesis. Hirschfeld and Hirschfeld (1912) tested serum and blood samples from anaphylactic guinea pigs for this type of substance but were unable to reach definite conclusions. Watanabe (1931) determined the amount of histamine like material from many guinea pig tissues before and after anaphylaxis and found significant changes especially in lung tissues. However the great variation found in control animals prevented them from concluding that histamine was released during the reaction. Failures by Rigler (1928) and Daly, Peat, and Schild (1935) to confirm Watanabe's indications were pointed out by MacKay (1938) to be due to the extreme variability in normal histamine levels of guinea pig lungs which may hold 7 to 88 mg. of histamine per Kg. of tissue.

Bartosch, Feldberg, and Nagel (1932) were the first to demonstrate histamine release by perfusing the lungs of a sensitized guinea pig with the

antigen. Except for chemical isolation of the active ingredient, a number of tests provided excellent proof that it was histamine. Daly and Schild (1934) confirmed these results and also showed that the active substance was inactivated by histaminase. For two lungs they found 1.7 to 12.8 μg. could be released by the perfusing antigen. Schild (1936) found similar results for passively sensitized animals. In these experiments a striking correlation was found to exist between the degree of bronchoconstriction and the amounts of histamine released.

Schild (1936) produced shock in two perfused guinea pig lungs with 1 and 10 μg. of egg albumen respectively, and by accounting for the amount of histidine in the antigen, being 2.5% of the egg albumen employed (Calvery, 1931), he showed that its decarboxylation during the perfusion could not account for more than 12% and 16% respectively of the histamine found in the perfusate. Thus he had proven that the histamine was derived from the lung and not from the antigen.

The following paragraph is taken from a review by Feldberg (1941)

> "There are responses such as that of the guinea pigs' uterus poisoned by histamine which are difficult to explain on the assumption that histamine is the sole factor responsible for all anaphylactic contractions but these instances do not invalidate the evidence that release of histamine is mainly responsible for the normal anaphylactic contraction of those muscles that are sensitive to histamine. The guinea pigs' uterus, for instance, contains between 9 and 20 μg/Gm tissue, 0.6 to 2 μg of which are given off if samples of the tissue are brought into a solution containing the antigen. A solution containing less than 0.1 μg/cc is usually sufficient to contract the muscle. The guinea pig's small gut has about the same histamine

equivalent on extraction but Schild (1939) could detect no histamine if samples of this tissue from a sensitized animal were brought into a solution containing the antigen. We do not know whether for anatomical or other reasons the released histamine is unable to diffuse into the bath fluid, or if there is a great quantitative difference in the actual release from these two tissues in which the anaphylactic reactions appear to be so similar. The fact that desensitization takes place indicates that the antigen-antibody reaction has occurred."

Adding to these remarks Schild and co-workers (Schild, Hawkins, Mongar and Herxheimer, 1951) say that histamine is probably the factor initiating the contraction during anaphylaxis, and suggest that the release of a large amount of histamine close to receptor groups offset any other influences on the muscle.

In guinea pigs, the acute anaphylactic shock with its fatal bronchoconstriction can be wholly imitated by the action of histamine which thereby is held responsible by most investigators. But histamine does not bring about the striking eosinophilia nor the chronic histological changes of the lungs caused by anaphylaxis (Kalos and Pagel, 1937). Feldberg (1941) considers these reactions to be direct effects of injury by the antigen and asks if peptone causes such changes. In partial answer, but in another species, Scroggie and co-workers (1947) found that platelets and leucocytes were greatly reduced in dogs after they had received injections of peptone, the reduction being accompanied by marked agglutination of the platelets. Eosinophilia was not specifically noted during this condition. Dworetsky and associates found that the great decrease in eosinophils due to cortisone or

ACTH treatment did not affect the severity of anaphylactic shock in guinea pigs. (1950)

Kellaway and Trethewie (1940) found that after perfusion of guinea pig lungs with antigen, an unidentified substance causing "slow contraction" of the suspended guinea pig ileum was in the solution as well as histamine. They identified the substance as being that which was also formed by the action of venoms on lipins. It may have been peptone. They concluded that the anaphylactic contraction of smooth muscle was in part due to liberation of histamine and probably, in part, also to the "slow reacting substance" which was liberated, not formed, in the antigen-antibody phenomenon. Campbell and Nicoll (1940) seem to have dealt with the same agent and discuss the possibility of its being a choline like substance. Recently Brocklehurst (1953) has conducted further tests to identify this slow-reacting substance. His work thus far has differentiated the substance from other tissue substances such as 5-hydroxytryptamine (serotonin) Substance P. and Bradykinin.

The possibility that heparin may have been liberated in the guinea pig anaphylactic reaction has been entertained by some authors who occasionally noticed an incoagulability of guinea pig's blood. (see Dragstedt for references, 1941).

Simon and Staub (1937) were apparently the first to detect increased quantities of histamine in the blood of intact, anaphylactic guinea pigs. Their animals were sensitized to and shocked with the chemical substance, arsenobenzene. Code (1939) repeated their observation with serum-sensitized animals and found the "blood histamine" to be increased of tissue histamine but Katz (1940) observed a release of cellular blood histamine into the plasma when antigen was added in vitro to sensitized guinea pig blood. According to Code(1952) this would probably mean particularly in Katz's experiment, that the histamine was released from either the granular series of leucocytes,

or from the platelets.

Schild (1939) found that temperature was a critical factor in the release of histamine in anaphylaxis, and that low temperatures prevented a maximal liberation. Release also occurred in two stages. An initial reaction during the first few seconds contact with the antigen requiring higher temperatures perhaps represented the actual release from the cells. A second slower phase able to procede at lower temperatures represented diffusion of the released histamine from extra cellular spaces into the fluid bath. These observations can be compared with those of Pickering (1952) who considered that the more immediate responses of anaphylaxis were due to antibodies acting on the outside layer of the tissue cells while delayed responses were due to cellular reactions some distance from the surface.

The desensitization which follows anaphylactic shock has been ascribed to either saturation of the antibodies (Ungar, 1944) or to the exhaustion of the histamine supply of certain cells (Dragstedt, 1943; Mead, Dragstedt and Eyer, 1937). Selye (1937) suggests the possibility of "adaptation" to the "alarming" stimulus.

(c) <u>The Release of Histamine in Rabbit Anaphylaxis.</u>

Arthus (1908-1909) made the first experimental study of anaphylaxis in the rabbit. The reaction reveals itself either as a local or a general manifestation depending on the route of administration of the antigen. The local reaction is termed the "Arthus Phenomenon" but this appears to take place without the participation of histamine. At the injection site during this manifestation an oedematous infiltration is followed by the appearance of a sterile abscess or gangrenous slough.

Intravenously, the antigen causes a rapid prostration of the animal with hyperventilation, elimination of urine and faeces, hyperaemia, and then anaemia of the ears. Heart failure and agonal convulsions terminate with the animal's death. The cause of death is not bronchospasm as in the guinea pig but a marked dilation of the right heart ending in cardiac failure (Auer, 1911). The dilation is caused by a spastic contraction of the smooth muscle of the pulmonary arterioles. (Simonds, 1919; Coca, 1919).

Many investigators have indicated that rabbit anaphylactic reactions are consistent with the pharmacological effects of histamine (Dale and Laidlaw,1910 - 1911; Bally, 1929; Rocha e Silva, 1940). The loss of "blood histamine" during anaphylaxis (Rose and Weil, 1939) from the rabbit's circulation appeared to contradict these early reports but later experiments (Barsoum and Gaddum, 1935;, Code 1937) showed that rabbit blood cells were actually the source of the histamine released during anaphylaxis. In this way they act much the same as dog liver cells and can be considered to be the "shock organ" of the rabbit.

Goetzl and Dragstedt, (1942) have shown that peptone reproduces anaphylactic symptoms in rabbits including the release of histamine. Although part of the "blood histamine" seemed to be due to mechanical removal of the leucocytes and platelets the main reaction is probably because of rapid diffusion of histamine from the cells to the plasma (Katz, 1940). In anaphylaxis, the decrease in leucocytes was also thought to cause this loss in blood histamine (Feldberg, 1941). According to Feldberg, the accumulation of leucocytes which occurs at the vascular wall during the anaphylactic contraction of the arteries (Abell and Schenk, 1938) should be regarded as a response of the leucocytes to the injury.

Abel and Schenck also showed that during rabbit anaphylaxis the leucocytes become sticky, adhering in clumps to the endothelium of liver vessels in many cases forming leucocytic emboli which obstruct them. This clumping was later shown to happen in the dog in a similar way (see Rocha e Silva for details 1950).

The only really positive evidence in sensitized rabbits concerns the release into the plasma of a considerable fraction of the cellular histamine when antigen is added to the blood (Dragstedt, Ramirez and Lawton, 1940; and Katz, 1940). Dragstedt et al, calculated that the in vivo release of histamine, after making in vitro observations, would be 0.1 to 0.3 mg/Kg. of body weight, an amount quite sufficient to account for all anaphylactic effects.

Kopeloff and Kopeloff (1941) and Kinsell and associates (Kinsell, 1941) have shown that a decrease in platelets is directly proportional to the gravity of anaphylactic shock in rabbits and also in monkeys. They correlated the rupture of platelets with the liberation of histamine.

Zon, Ceder, and Crigler (1939) have made the most conclusive studies on the histamine content of rabbit platelets. After fractional centrifugation of the blood there was a high correlation between the number of platelets in suspension and the amount of histamine in the fluid. Anti-platelet serum injected into rabbits caused decisive but not parallel drops in both the number of platelets and the blood histamine concentration.

Further work by Rose (1941) has shown that in rabbit anaphylaxis a decrease in histamine also occurs in such tissues as the lung, liver and spleen and others have indicated that the rabbit lung released a small amount of histamine (Dragstedt et al, 1940, Katz, 1941), Rose and Leger (1952)

have lately presented evidence that the rapid disappearance of histamine in plasma and also in other tissues may be due to the increased activation of histaminase. Earlier Dragstedt reported complete failure to reduce the histamine content of tissues with histaminase (Dragstedt, 1943).

Preservation of platelets by oxalate and citrate has been shown to prevent the release of histamine from rabbit cells. (McIntyre, Roth, and Richards, 1949).Heparin prevents this release to a smaller extent (Dragstedt, Wells, and Rocha e Silva, 1942). According to Rocha e Silva's concept (1952) these substances act by preventing the release of a certain factor from platelets that is necessary for anaphylatoxin formation.

While Rocha e Silva (1950) agrees that the white blood elements are the most important single constituent in rabbit anaphylaxis he postulates that their role in other species is one of providing, subsequent to their disintegration, an activator of the serum protease which causes the release of histamine and other anaphylatoxins. Evidence for the presence of such an activator has been provided by Scroggie et al (1947). They have found that activation of serum protease in peptone shock is a step in the release of histamine by rabbit cells, and it is possible that this mechanism also occurs in anaphylactic shock. On the other hand, McIntyre, Roth and Sproull (1952) did not detect the presence of an activated plasma protease either during fatal <u>in vivo</u> shock or in <u>in vitro</u> release of histamine from rabbit cells. Activation of the plasma protease by streptokinase was not followed by other anaphylactic symptoms <u>in vivo</u> or <u>in vitro</u>. The plasma protease theory of anaphylactic shock was therefore not tenable.

(d) <u>The Release of Histamine in Anaphylaxis of Other Species</u>.

A great number of experiments have shown that anaphylactic shock

in some other species probably causes the release of histamine. However all evidence to date seems to be circumstantial and the release of histamine during anaphylaxoid states in these animals has not definitely been shown.

In experiments on the rat Kellaway (1930) showed that histamine probably does not take an active part in the anaphylaxis of that species. The isolated uterus of a sensitized rat responded by contraction to the antigen but histamine in moderate doses produced only relaxation. The relative insensitivity of the rat to both anaphylaxis and histamine is probably significant. The lethal intravenous dose for the white rate is 300 mg/Kg (Voegtin and Dyer; 1924) and it is doubtful that the rat could release that much histamine during anaphylaxis. Suden, (1934) has observed blood pressure falls during rat anaphylaxis similar to that caused by histamine. Likewise the mouse is extremely resistant to any sensitization and many have attributed the great difficulty in inducing anaphylaxis in them to their low sensitivity to histamine (Mayer, 1950). Mayer states, "Since the total histamine content of the normal mouse is approximately 10mg. of histamine per kg. of body weight and since mice present the first signs of histamine shock only when 100 or more mg. of free base are injected, it is rather improbable that histamine is involved in mouse anaphylaxis".

The studies of Saunders (1951) indicate that the mouse has an extremely active adrenal gland and to this is attributed the resistance of the mouse to both anaphylactic and histamine shock. Lethal doses of histamine for rats had previously been shown by Wyman and Suden (1937) to be decreased in adrenalectomized animals. Also, Perry and Darsie (1946) had shown that adrenalectomy in the mouse greatly enhanced the possibility of evoking anaphylaxis. Thus Saunders' work indicates that histamine may possibly be the mediator of anaphylaxis in the mouse.

Cats have been sensitized only with difficulty by a number of workers (See Dragstedt for refs., 1941) but even though effects analogous to those caused by histamine occur during the shock it has not been proven that histamine is released. Some of the authors have noticed an increased clotting time of the blood in cat anaphylaxis.

(e) The Release of Histamine in Human Anaphylaxis:

Anaphylaxis is a class of hypersensitiveness confined mainly to lower animals. In man this term is not usually employed having commonly been replaced by the word "allergy". However, this does not carry the meaning that "anaphylaxis" holds for animals because "allergy" is also employed to define the abnormal reaction of tissues to physical and chemical stimuli. The distinctive type of human hypersensitiveness corresponding to anaphylaxis is designated as "atopy" and includes hay fever, asthma, atopic dermatitis and urticaria.

It is a rather well known fact that histamine administered either locally or generally produces many of the effects of atopy. This was realized quite early by Lewis (1927) who found evidence for the release of histamine in many skin reactions. He said "it would be gratuitous here to postulate more than one substance (released) since one suffices". However the actual release of histamine has only been demonstrated in a few of these reactions. The reason for this has either been due to the lack of a sensitive method for determining histamine or because of the few opportunities available for the clinician to take blood samples before and during spontaneous asthmatic or other atopic attacks. Data is usually obtained from laboratory investigations on isolated blood or tissue samples where the patient is not exposed to the dangers of an induced general reaction.

In humans the reaction may appear in any number of forms and this is governed by the antigen and the route of its entry. Most charactistically the allergic response is a localized tissue oedema or increased activity of smooth muscle. Thus in the nose there is obstruction and sneezing; in the bronchioles there is cough, dyspnea and wheezing; and in the intestine colicky pain and diarrhea may result. Skin reactions are red, itchy or burning areas, not unlike those caused by histamine. Frequently blisters appear. In the reaction known as "serum sickness" the symptoms of urticaria fever and joint pains persist for several days.

For other details on allergens, symptomology and routes of sensitization, the reader is referred to the monograph by Feinberg (1946).

The first definite suggestion that human hypersensitiveness, hayfever, was related to anaphylaxis in animals was made by Wolff-Eisner (1906). Soon it was suggested that asthma and other phenomena could be similarly explained and the field was rapidly over run with a list of conditions having the now-familiar symptoms. However, proof of their relationships through histamine has only partly been realized.

Lewis and Grant (1925-1926) found that local reactions in a person sensitive to fish extracts were identical with those caused by histamine but this did not constitute sufficient proof that histamine was involved in the reaction. Cerqua (1936) was the first to demonstrate that blood histamine increased during urticarial attacks. Soon many others (Tarras-Wahlberg, 1937; Capps and Young, 1940; Randolff and Rackemann, 1941) reported finding an increased level of blood histamine associated with an exacerbation of symptoms in allergic patients. Most recently Adam, Hunter and Kinnear (1950) have made observations on the urinary excretion of histamine in urticaria. Their evidence supports the conception that his-

tamine is either liberated or newly formed in this condition but because of the low sensitivity of their methods no definite conclusions can be drawn.

Katz and Cohen (1941) reported that the addition of the specific allergen to the blood of allergic patients caused a release of histamine from the cells to the plasma. The increase of plasma histamine was 160-900%. Later, Katz (1942) reported that the application of the specific antigen to the denuded skin of allergic patients resulted in the sharp increase of histamine released from the area tested. He also noted the parallel release of a "slow reacting substance". Although there seems little reason to doubt that the active substance actually was histamine in none of these cases could the identification be clearly established.

Recently Schild et al (1951) have probably made the most significant studies of human histamine release. They investigated "whether isolated bronchial rings, obtained at operation from a patient with asthma, would contract in the presence of specific antigen and be desensitized after the first addition of the antigen; whether histamine would be released from bronchial and lung tissue in the course of the reaction; and whether allergic broncho-constriction could be suppressed by anti-histamine drugs". Their results showed that the bronchial preparation was desensitized after a single dose of one antigen although it responded to a second specific antigen. Histamine was released during the response. Antihistaminic drugs in extremely high concentrations (10,000 times those required to antagonize the effects of added histamine) antagonized the response of the allergic tissue to antigen. The necessity for this high concentration of antihistamine, they explain, was probably due to histamine "released in concentrations

which are presumably very large locally, requiring large concentrations of antihistaminic drug to antagonize it."

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In this section the author has attempted to provide a basis for the comparison of other conditions in which histamine is released to anaphylaxis. In doing so it has been shown that in the phenomenon of anaphylaxis in various species there are a great many similarities. One of the most obvious of these is the release of measurable amounts of histamine. This has been shown to occur in every species tested with the possible exceptions of the mouse and rat. The release of one or more "slow reacting" substances has also been shown to occur in most species during anaphylaxis. The possibility that heparin is released in this condition in the dog and guinea pig has been shown by the experiments of some authors. The participation of certain blood elements in the reaction has been extensively studies and most certainly occurs in the dog and rabbit. For several species, evidence has been produced to suggest the activation of a serum or tissue proteolytic enzyme and its participation in the anaphylactic reaction.

Besides these factors the symptoms of anaphylactic shock can sometime be compared from species to species. The exhibition of these same symptoms following the injection of histamine provides another reason for believing that the anaphylactic syndrome in many species is caused by the release of large (?) quantities of histamine.

In almost every instance where an animal or isolated tissue has recovered from a profound anaphylactic reaction, subsequent injections of the antigen produce no effect. This "refractoriness" usually passes off, in whole animals, after a few days. The reduction of anaphylactic symptoms in many species by other substances, especially the antihistamines is also suggested to be due to a similarity, in the species, of the mechanism of anaphylaxis.

3. THE RELEASE OF HISTAMINE IN TRAUMA

This section is separated from the following ones by arbitrarily assuming in them that a number of influences may cause the release of histamine without damaging the tissue in question. In many instances this difference is an extremely small one and will seem to apply in name only.

(a) Histamine Release by Mechanical Injury:

Mild tissue injury such as stroking the skin was shown by Lewis and Harmer (1926) to cause the release of a histamine-like vasodilator substance. The flushing of the face and hand and also the skin temperature rise were similar to those caused by histamine.

Even though injury of the cell by sand-grinding tissues into very small particles liberates histamine, Trethewie (1938) has shown that the smallest cell fragments may still retain much of their histamine content. Thus the histamine cannot be regarded as simply existing in a freely diffusible form in the cell.

It is important to notice that not even in anaphylactic shock nor by the action of "histamine releasers" (next section) is more than a certain percentage of tissue histamine released (Mongar and Schild, 1952). If these reactions are regarded as being similar to each other causing a limited amount of cellular injury then only complete cell destruction would release all the cell histamine.

With reference to the rabbit blood cells, Code (1952) states that they are extremely susceptible to mild trauma. The following quotation (Code, 1952) illustrates the dangers involved in taking rabbit blood samples:

"The blood should certainly not be exposed to surfaces upon

which the platelets or white cells may adhere. Dry wettable surfaces such as those offered by dry syringes, dry tubing or glassware cannot be used. All surfaces touching the blood must be non-wetting. Even strands of fibrin or small bubbles in needles or syringes may alter the distribution of histamine by providing surfaces to which the cells may adhere. Any shaking of the blood must be avoided. The plasma, too, should be separated immediately after the blood is drawn since the distribution ofhistamine may be altered by standing. Chilling the blood may also aid in preserving the normal distribution".

Code (1937) could not find any difference in "total blood histamine" after the clotting of rabbit blood. During the process of coagulation, however, there was a shift of histamine from the cells to the serum.

A number of papers and reviews (See Best and McHenry, 1931) have emphasized the similarity between wound shock and histamine poisoning. Extracts of lacerated, autolysed or bruised tissues have been shown to produce the same histamine shock-like condition. Bayliss and Cannon (1919) were able to demonstrate the presence of a histamine-like substance in the blood stream following the crushing of denervated limb muscles. However, other evidence has indicated that the fall of blood pressure in traumatic shock in animals can be attributed to the loss of blood and tissue fluids into the damages tissue and not to the absorption of toxic substances.

(b) Release of Histamine by Burning

The release of histamine in cellular injury by burning had been studied by a number of investigators. Lewis and Grant (1924) were the first to make a study of the process but they could only postulate the

release of a histamine-like substance. A minute skin burn was shown to reproduce perfectly the effects of intradermically injected histamine.

In severe burns in humans, Rose and Browne (1942) found a definite increase in blood histamine within one hour after the burn. Then, with toxaemia and oedema the histamine level decreased. They also noted a correlation between the degree of the burn and the histamine level of the blood.

Harris, (1927) has shown that the burning of cats skin caused a reduction in skin histamine content. The loss of histamine only occurred subsequent to oedema formation and was not observed if the skin area was removed immediately after burning. Kisima (1938) showed that dogs would not suffer from burn shock if the burned area was removed directly after injury. This treatment, as shown by Dekanski (1945) prevents the effects of liberated histamine. Dekanski demonstrated that extensive cutaneous burns in mice increased the formation of new skin histamine very rapidly. In 10 minutes the histamine content of the whole mouse was doubled. However, he has not ventured to guess the source of this new histamine. Perhaps it was the result of histidine decarboxylation.

In another paper Dekanski (1947) states that only with 60°C burns is the skin histamine increased. With 80°C and 140°C burns the histamine present slowly disappears. He believes that the histamine formation "depends on the activity of irritated living cells". Dead cells he noted, formed none and whatever histamine they did contain was lost and destroyed or reabsorbed.

Histamine-like-activity has also been found in the perfusion fluid of the isolated burned cat heart (Bennet and Drury, 1931) and in venous perfusates of hind extremities of animals that had received skin burns (Nagamitu, 1935).

Further work by Dekanski (1949) indicates that the effects of burning to release histamine may be mediated by the substance "leucotaxine" (Menkin, 1937) This substance, a polypeptide, having 8-14 amino acid residues and a molecular weight of about 1500 (Miles and Miles, 1952) has just lately been shown to cause the release of histamine (1953; personal communication from A.L. Grossberg). A short time earlier, Beloff and Peters (1944-1945) found that after burning the tissues contained a "liberated enzyme" resembling trypsin. This they claimed was the "burn toxin". It is doubtful that this "proteinase" has any relation to leukotaxine which possesses no enzymatic activity. Feldberg (1941) has noted that any factor such as heat which alters the structure of either the lipins or the proteins of the tissues would probably release histamine. It is also to be remembered that just such a technique has been employed as a method in the extraction of tissue histamine for a number of years.

(c) Histamine Release by Irradiation

Lewis and Zotterman (1926) were the first to suggest that the vasodilatation in human skin subsequent to exposure to sunlight or ultraviolet light might be due to histamine. With a very intense localized exposure they were able to follow the course, through the lymphatic channels, of the vasodilator substance elaborated in the skin, by charting the dilatation of the overlying skin vessels. The same dilatation would also occur with administration of histamine under the skin.

In Lewis' monograph (1927) further evidence links the effects of ultraviolet light, X-Rays and radium emanations with those of more quickly acting stimuli. In all these instances the vessels were found to be left in a common state of refractoriness to histamine and irresponsiveness to vasoconstrictor substances.

The production of histamine from histidine by ultra-violet light at 250 mμ was demonstrated by Ellinger (1928) who believed that histamine was formed by the decarboxylation of histidine. He also believed that histamine did not exist in the tissues as such but only occurred with the decarboxylation. However, Bourdillon et al (1930) produced evidence that whereas ultra-violet light of a wave length under 265 mμ could produce histamine from histidine, such light could not penetrate human skin and was also not present in sunlight. These authors therefore held it more probable that the irradiation liberated preformed histamine.

Summing up the evidence Laurens (1941) states: "From the amount of energy constituting the minimal erythemal dose and the absolute quantum efficiency of the total photochemical changes in typical proteins and the concentration of protein decomposition products, the direct photochemical production of H-substance from the proteins of the stratum mucosum is reasonable from a quantitative point of view".

(d) Histamine Release by Chemicals

Lewis (1927) found that whatever the nature of the provocative injury-mechanical, thermal, electrical, chemical or photochemical, the reaction was always the same. He believed that this reaction, the triple response, was caused by a chemical stimulus. However, he had no proof for his belief. His only data consisted of a long list of substances which reproduced the effects caused by histamine. These were hydrochloric acid, sodium hydroxide, silver nitrate, copper sulphate, mercury bichloride, and alcohol. These substances were thought (Rideal, and Schulman, 1939; and Feldberg, 1941) to act on the lipoproteinic structure of the cell in which the histamine was anchored. It should be remembered that some of these substances are still used in the quantitative estimation of tissue histamine.

Best, Dale, Dudley and Thorpe (1926) have given an account of extraction procedures using a few of these materials.

Many irritants have been claimed to cause histamine release when applied to the skin. Lewis (1927) again compiled such a list which included mustard, mustard gas, cayenne pepper, cantharidin, and chloroform. He claimed that the symptoms observed were all due to tissue injury. Later xylene, croton oil, turpentine and colchicine were found to produce similar effects (See Selle 1946 for references). Selle has compared the reactions due to these irritants to the action of various venoms, trypsin, allergic manifestations and ultra-violet irradiation.

Bartosch (1936) was able to show that small amounts of heptane, octane, and benzol, administered to guinea pig lungs by way of the trecheal cannula, caused a release of histamine which was detectable in the perfusate. Perfusion of a rabbit's ear with these substances also caused a release of histamine.

The irritating vapors of ammonia, acrolein and toluol were similarly shown to release histamine from guinea pig lungs (Garan, 1938). More recently, Schild (1949) has demonstrated that ammonium chloride will liberate histamine quantitatively from striated muscle if the pH is high enough to liberate free base.

All the above mentioned irritants are believed to cause the release of histamine by cellular injury. Whether a specific mechanism underlies this effect has not been demonstrated. Several investigators (Astrup, 1950; Kaplan, 1944) have reported that chloroform is able to activate serum fibrinolysin but this observation has not been extended to include other irritants.

There is a possibility that another mechanism takes part in the inflammation stage of irritation. Rocha e Silva (1944) has mentioned that increases in skin histamine may be due to the collection of platelets at the site of inflammation. This suggestion seems to be a sound one because Loomis, et al (1947) have found that a platelet factor activates the enzyme "fibrinolysin".

(e) Histamine Release by Venoms and Toxins

Kellaway (1929) was the first to mention the possibility that the stimulating action of snake venoms on the guinea pig uterus may be mediated by the same factor as was produced by the anaphylactic reaction. Histamine, however, was discarded since the venoms contracted the rat's uterus - an action that histamine does not have.

About the same time Essex and Markowitz (1930) compared the effects of crotalin (rattlesnake venom) shock to anaphylactic shock in dogs. They found such similarities as, a sharp fall in blood pressure, and initial constriction of splanchnic viscera followed by engorgement, a loss of blood coagulability and a rise in bladder pressure. The triple response could also be obtained with crotalin as could histaminic or anaphylactic-like effects on isolated guinea pig uteri or bronchioles. Except for isolation of the active agent producing these effects in both conditions the comparison between crotalin and anaphylactic shock was almost complete.

Probably the earliest, and most complete, report of histamine release by venoms was made by Feldberg and Kellaway (1937). They perfused the lungs of cats and guinea pigs with the venoms of three varieties of reptile (copperhead, cobra and rattlesnake) and found a histamine-like substance had been released. The release was accompanied by a bronchoconstriction and swelling of the lung - a condition also noticed with histamine or specific antigens.

Depending on the dose of the venom almost all of the histamine-like material could be perfused from the lung. In the case of the rattlesnake venom, an immediate proteolysis was followed by destruction of the lung tissue. A "slow-reacting substance" was also liberated during the action of the venoms.

These workers (Feldberg and Kellaway, 1938) showed that the release of histamine was probably mediated by "lysocithin", a mixture of lysolecithin and lysocephalin produced by the action of venoms which contained a lecithinase. Lysocithin could be released from venom-perfused monkey livers without the liberation of histamine. Subsequent treatment of dog livers with this monkey lysocithin released histamine just as the pure venom would. The formation of "slow-reacting substance" was also thought to be due to the action of lysocithin. (Feldberg, Holden and Kellaway, 1938). An alcoholic extract of lecithin incubated with cobra venom had two main properties - a lytic one and a smooth-muscle-contracting one. The latter was due to "slow-reacting substance" and they found that it imitated the actions of venom and anaphylaxis more closely than did histamine. The output of histamine and "slow-reacting substances" were found to bear no relation to each other but usually histamine was released first.

Another property of lysocithin was believed by Feldberg (1940) to be its ability to produce a long lasting output of adenalin from the adrenal glands. He suggested that the adrenalin was freed by the direct lytic action of this venom-formed material and histamine was not an intermediary in the reaction. This suggestion is in opposition to the observations of Burn and Dale (1922) who showed that when histamine was injected into the adrenal artery of a cat there resulted a transient rise in blood pressure. Also anyone who has made very many histamine injections will vouch for the

belief that adrenalin is apparently released even when histamine is given by the femoral route.

Feldberg and Kellaway(1937) found that the lungs of young kittens contained little histamine and showed less symptoms from snake venoms than did older cats lungs in whom the histamine content was high. However, the cellular injury was also greatest in the lungs of the older cats and this suggested that the release of histamine was in the same way connected to the degree of cellular destruction.

Starting with observations on snake venoms Rocha e Silva (1938,1940) found that the effect of crystalline trypsin resembled those of histamine, venom or anaphylaxis and caused a release of histamine when perfused through guinea pig lungs. The mechanism seemed to act in two phases. Digestion of the protein would first lead to destruction of the normal lipo-protein structure, and then, when peptones were formed by the tryptic digestion, a further release would be initiated. In venom poisoning a spolit product of lipin and proteolytic digestion could also cause the release of histamine. (Feldberg 1941). The venoms act by splitting off oleic acid from lecithin and forming the lytic substance lysocithin.

Bee venom has been shown to act in much the same way as snake venom (Feldberg and Kellaway, 1937-1938). A guinea pig lung perfused with the equivalent of 5 bee stings increased the output of histamine from 0.01 µg/min to 0.11 mg/min. 75% of the lung histamine was removed after 3.5 hours of perfusion. In addition, this venom had both fast and "slow-reacting" direct effects on the isolated intestine.

In order to reconcile the mechanisms occurring in venom shock and anaphylactic shock, Feldberg (1941) postulated that the antigen might affect the sensitized cells by destroying anti-tryptic factors, allowing

cellular trypsin to act. Thus the release of histamine together with the formation of peptones from the tissue proteins could account for all the known smooth muscle stimulating effects not only of venoms but also of peptone and anaphylaxis.

Staphylococcal toxin, prepared in agar nutrient broth was shown by Feldberg and Keogh (1937) to have a histamine releasing action by cellular injury, in perfused cat and guinea pig lungs. This particular bacterial toxin was chosen because it was known to have a shorter latent period in the production of symptoms than other toxins. (Kellaway, Burnett and Williams, 1930). The histamine was released only after a long latent period of between 10 and 40 minutes and amounted to values ranging between 4 and 15% of the total lung histamine. The authors suggest that this mechanism of histamine release is probably involved in the action of other bacterial toxins.

A different type of bacterial reaction is observed in the Schwartzmann phenomenon. If a rabbit is given an intradermal injection of a small amount of filtrate from a bacterial culture, and 24 hours later receives an intravenous injection of the same filtrate or even of a filtered culture of certain bacteria different from those used for the intradermal injection, a haemorrhagic lesion appears at the site of the latter. Rocha e Silva and Bier (1938) found that fragments of skin removed from the haemorrhagic sites usually contained more histamine than normal speciments. They believed that histamine was liberated during this phenomenon and acted as an adjuvant enhancing fragility and permeability of capillaries, thereby producing haemorrhagic areas. Recently Rocha e Silva (1952) found indications that the presence of platelets was an important factor in the development of this experimental purpura.

The significance of this detail will be seen later in connection with the activation of histamine-releasing fibrinolysins. Schwartzmann proposed the concept that some inflammatory and necrotic manifestations of various infectious diseases, such as typhoid and smallpox, might be based on this type of hypersensitiveness.

4. HISTAMINE RELEASE BY ENZYMES

Many actions of histamine-liberating substances or mechanisms have been at least partly explained by the postulation or demonstration of enzymes taking an active part in the process. This section will deal with that aspect of the reaction more completely. Because of the great number of papers written on the enzymatic systems involved in the release of histamine, the author has restricted himself to a short review of some of the major aspects of the problem. The early part of this phase of histamine release has been summarized by Dale (1920) who mentions the work of Jobling (1914) and Bronfenbrenner (See Bronfenbrenner for refs., 1944). They described a number of chemical and physical agents which weaken or destroyed the "antitryptic" power of normal serum. The tryptic ferment, normally kept in abeyance, initiated a self-digestion of serum proteins. By removing the anti-trypsin, the antigen-antibody union determined the onset of protein cleavage. The toxic cleavage products were then produced from the animal's own blood proteins. Abel and Kubota (1919) also suggested that the intracellular formation of histamine by protein digestion caused the anaphylactic symptoms, so similar was the reaction to that produced by directly injected histamine.

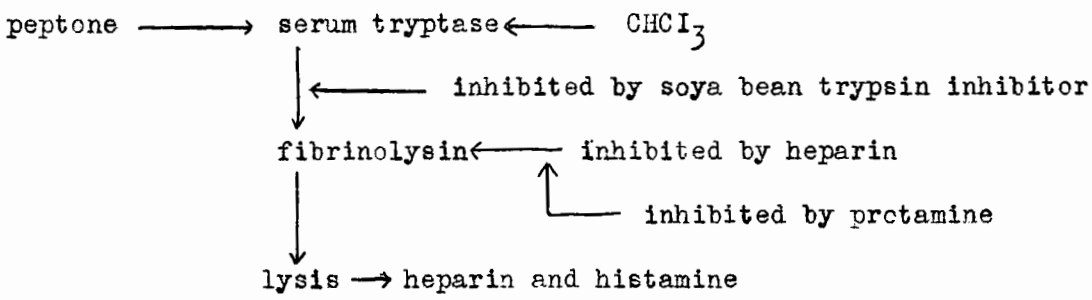
Many others have mentioned that a proteinase constituted the common factor behind the liberation of histamine in various procedures. (Beloff and Peters, 1944-1945; Tepperman, Engel, and Long, 1943) but the problem did not seem to be so simple as a mere tissue digestion and disintegration.

Rocha e Silva and Andrade (1943) found that the histamine-liberating power of papain, a vegetable "pepsin" much like intracellular cathepsins, ran parallel with its' capacity for attacking the arginine-amide linkage. They concluded, quite naturally, that histamine in the cell, was bound by an amide type linkage with arginine or lysine. Extending these findings to living cells, (Rocha e Silva, 1944), cathepsin 2 was postulated as being the proteolytic enzyme which might liberate histamine when its activation took place, for example in anaphylaxis. Rocha e Silva and co-workers (Rocha e Silva, Andrade, and Teixeira, 1946; Rocha e Silve and Teixeira, 1946) have also found ample evidence that activation of proteolytic enzymes takes place in anaphylactic and peptone shock.

Rocha e Silva (1950) considers that the protease system is probably the missing link in the chain of events leading to the release of histamine. In such conditions as anaphylactic or peptone shock in the dog the presence of the protease can only be detected, after the concomitantly released heparin is neutralized with protamine (Jaques and Waters, 1941). The protease, fibrinolysin, cannot be detected in the blood after several doses of antigen or peptone, and therefore, desensitization might depend on either an exhaustion of the mechanism leading to fibrinolysis or on the discharge of an antifibrinolytic agent which would block the first wave of fibrinolysis. The fibrinolytic activity is not blocked if heparin can

be excluded from the system. If a"thoracic"dog is used the release of heparin does not take place and so the presence of the protease can be demonstrated much more readily.

In peptone shock in the anterior or thoracic dog, the following events have been shown (Scroggie, Jaques and Rocha e Silva, 1947) to take place.



The soy bean trypsin inhibitor was first isolated by Ham and Sandstedt (1944) and was later shown to act by forming almost instantaneously and irreveraible stoichiometric compound with trypsin (Northrop, Kunitz, and Herriot, 1948). The inhibitory action of the soy-bean factor has been shown by Tagnon and Soulier (1946) to extend into systems where the plasma enzyme was being activated by either chloroform or streptokinase.

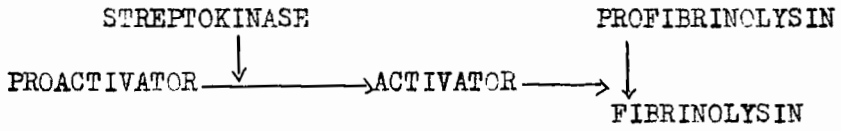
McIntyre, Roth, and Sproull (1950) have produced evidence to indicate that the soy bean inhibitor does not exert its effect in the anaphylactic reaction. Using a method similar to that of Scroggie, Jaques and Rocha e Silva (1947), they found that the soy bean preparation did not inhibit the release of histamine from sensitized rabbit cells treated with the specific antigen. This view seems to be supported by the investigations of Ungar and Mist (1949) who found that the activation of the serum fibrinolysin by $CHCl_3$ or streptokinase differed from that caused by the specific antigen, peptone and certain polypeptides in that activation by the latter agents required the presence of a certain

heat labile serum constituent. This factor was assumed to be a fibrinokinase and the possibility was suggested that the protease activated by chloroform or streptokinase was not indentical with that activated by the other substances.

The difficulty of preventing anaphylactic reactions with the soy-bean inhibitor may probably be due to the size of the molecule, which prevents its diffusion from the blood stream to the tissues. Thus when the anaphylactic reaction occurs, no SBI would be in the vicinity to prevent the activation of the tryptase.

The earliest reports had indicated that certain streptococci produced a fibrinolytic enzyme (Garner and Tillet; 1934). However this was later shown to be an enzyme activator which produced a "lytic factor" from an inactive serum precursor (Nilstone, 1941; Christensen, 1945). The lytic factor is now known most widely as "plasmin" or "fibrinolysin".

Most recently Mullertz and Lassen (1953) have shown that streptokinase, the streptococcal agent, transforms a proactivator in blood to an activator which in turn converts pro-fibrinolysin to fibrinolysin



The "anaphylatoxin" which caused so much trouble in the early manuscripts seems to be the same as "fibrinolysin". All the activators such as kaolin, chloroform, barium sulphate, agar, starch or inulin must act similar to streptokinase in producing the anaphylactic-like symptoms. (Rocha e Silva, 1951).

However the complexity of the problem has been shown to be greater than the above statement seems to indicate. Vinazzer (1951) considers that an anti-fibrinolysin is present in the plasma and can be inhibited by heparin to some extent. Also, barium sulphate was shown to absorb this anti-fibrinolysin (A.F.L.) and thereby release the active fibrinylysin (F.L.). This is at least partially supported by the work of Kaplan who found that A.F.L. and F.L. combine rapidly and specifically in multiple proportions. (Kaplan, 1945). Schmitz (1937) has suggested that the lysis of the fibrin clot after coagulation is initiated by a similar fibrinolysin activating mechanism. This was indicated in an article by Iyengar, (1942):

"According to Schmitz, during clotting the trypsin-inhibitor compound is absorbed on the fibrin clot. The kinase inhibitor compound which is also present in plasma is broken up into its constituents with the result that only kinase, which is probably of a protein nature, gets absorbed on the clot while the inhibitor which is a polypeptide of low molecular weight remains in the serum".

The recent work of Ratnoff (1953) has shown that this may be very close to what actually takes place. However he found evidence that the clotting process accelerated the formation of fibrinolysin from profibrinolysin and did not, as one might expect from the above description, seperate F.L. from an F.L.-A.F.L. complex.

Other experiments indicate that many of these factors have their origin in the white cell layer of the blood. Johnson and Schneider (1953) have found that 70% of the blood A.F.L. is found in platelets and they believe that this is the source of all blood A.F.L. Christensen and McLeod (1945) and Christensen (1946) have shown that the fibrinolytic

pro-enzyme (tryptogen, plasminogen or profibrinolysin) is activated by a platelet factor having the properties of a kinase. These studies have provided very good evidence that both F.L. and A.F.L. are as real as the proenzyme P.F.L.

The activation of fibrinolysin, then, has been shown to be initiated by two distinct processes: kinase is activated either by separating the A.F.L. - F.L. complex or by the formation of kinase from profibrinolysin. In the former category seem to belong such activators as barium peptone and the antigen-antibody complex. The other group seems to include the proenzyme activators chloroform and streptokinase, and possibly clotting. However there is little conclusive evidence on either of these points. The one important fact that remains is that both types of fibrinolysin-activating systems do exist.

Ungar (1951) and Ungar and Damgaard (1951) have recently shown in experiments on rats that some of the factors, participating in the proteolytic mechanism, are under endocrine control. ACTH, cortisone and Splenin A accelerated the inactivation of fibrinolysin by antifibrinolysin: thyrotropin, somatotropin, thyroxin, desoxycorticosterone acetate, and Splenin B diminished this action. The spleen was shown to be the controlling organ in the action, exerting its effect via the substances Splenin A and B. In splenectomized animals, only Splenin A and B could produce an effect. Taking certain portions from the above mentioned papers the scheme in Figure 1 has been adopted, and can be used to illustrate many of the factors participating in histamine release.

The many factors mentioned in the above section indicate the complexity of this vast problem. It is hoped that it can be read with understanding but the author has not attempted to explain the great mass of investigation on the subject.

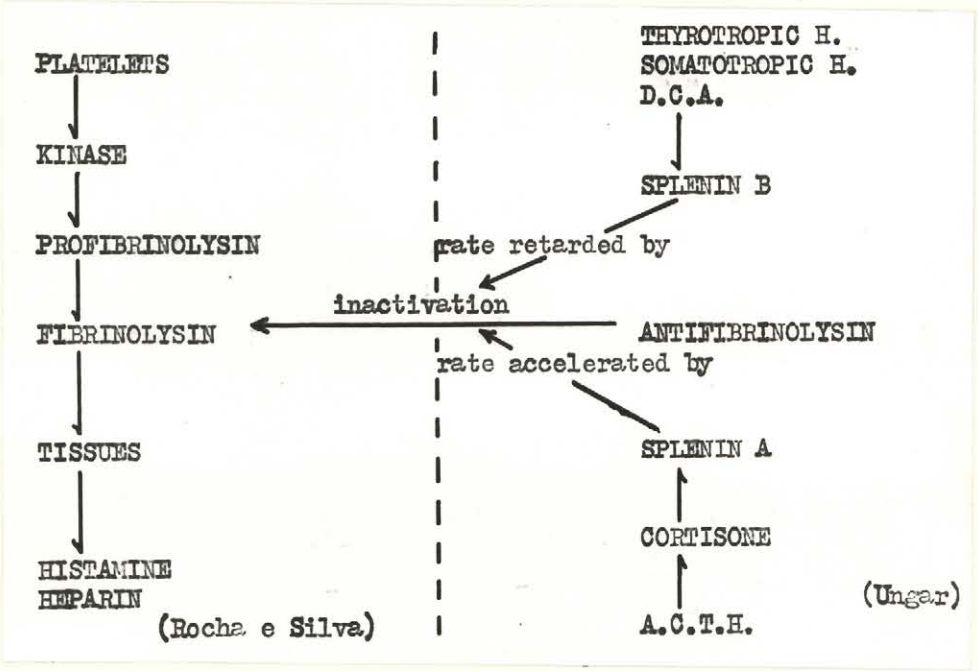


Figure 1.

A scheme for the participation of endocrine factors in the mechanism of histamine release.

A very good review on the nomenclature of parenteral proteases by Ferguson (1948) will be helpful to the interested reader.

The part played by enzymes in the release of histamine is further discussed at the end of this chapter.

5. THE RELEASE OF HISTAMINE BY "HISTAMINE LIBERATORS"

Thus far, histamine release has been shown to take place only after a number of injurious stimuli. In the previous section the activation of tissue proteases or various enzymes after cellular destruction was suggested to be the manner by which harmful procedures produced their effect. A growing number of chemical compounds are believed to cause the release of histamine without damaging tissue cells. These have been named "histamine liberators".

Although the expression "histamine liberator" was just recently coined, (MacIntosh and Paton, 1949), compounds like peptone, previously well known as having this effect, have also been included in the category. Actually, the term is a rather vague one and the only prerequisite for acquiring this designation, besides causing the release of histamine, is that the substance must cause no gross structural change during the time of its application. However, one other feature common to most "releasers" thus far studied is that they all contain one or more basic radicals in their structure. Tween 20 and dextran are exceptions to this "rule".

It seems strange that the term "histamine liberator" has remained so long in use. Even at the time of its inception a "histamine liberator" was also known to have other actions. Although the term now in use certainly describes the most important action of these substances probably "anaphylactomimetic" is a more suitable name. The appropriateness of

this designation will become evident in the following section.

(a) Peptone

As already has been suggested, peptone was the first "histamine liberator" shown to possess that action. In 1937, Feldberg and O'Connor, noted that the perfusion of cat and guinea pig lungs with solutions containing peptone released 10% and 3%, respectively, of their histamine content. About the same time Dragstedt and Mead (1937) detected a histamine-like substance in the blood of peptone-shocked dogs.

The great similarity between the effects of peptone and histamine were first pointed out by Dale and Laidlaw in 1910. The only difference existing between these two agents was that in peptone shock the blood became incoagulable. This was also known to occur in anaphylactic shock. In 1938, Waters, Markowitz, and Jaques were able to show that the incoagulability of the blood in both conditions was due to released heparin. This step narrowed the gap of difference between the two reactions in dogs considerably.

The release of histamine from the blood cells of rabbits, guinea pigs and rats with peptone as shown by Ungar (1944) paralleled that caused by antigens as demonstrated by Katz (1940). In guinea pigs, Ungar (1947) found a further similarity in the effects of anaphylactic and peptone shock. He noticed that serum protease was activated in both states. It is now accepted that the symptoms apparent in the two conditions are largely due to liberated histamine. However, no technique has yet shown just how each process is initiated.

Among many resemblances between peptone and anaphylactic shock, Beidl and Kraus (1909) remarked that in both reactions the polymorphonuclear leucocytes disappeared almost entirely from the blood. Dale

and Laidlaw (1918) found that this also occurred in histamine shock.

Rocha e Silva (1950) has recently described this similarity in a more enlightening manner. Summarizing his statements: Initially, a constriction of the hepatic vessels transforms the liver capillaries into a filter for the agglutinated platelets and leucocytes collected in the liver. After a brief latent period the platelets "explode" and then histamine and heparin are released. The clumping of platelets seems to be mediated by a glycogen-like polysaccharide but this has not yet been clarified.

The inhibitory action of heparin again closely associated the mechaniom of peptone shock with that of anaphylaxis. The following quotationfrom Rocha e Silva makes this clear: "------ this effect of heparin protecting the clumps of platelets against destruction and at the same time preventing activation of the fibrinolytic enzyme of the blood, constitutes good evidence that products derived from platelets might be concerned with the activation of this fibrinolytic enzyme of the blood during anaphylactic and peptone shock."

This protective action of heparin has also been noted by Dragstedt Wells, and Rocha e Silva (1942) for peptone, trypsin and antigen on normal or sensitized rabbit cells and by Macht (1944) for trypsin and papain in intact animals.

Other studies have indicated that the refractory state which follows both anaphylactic and peptone shock are due to the same cause (Hanzlick, 1942). The desensitization which follows anaphylactic shock is generally ascribed to the saturation of antibodies, but with peptone such an explanation is impossible because peptone is non-antigenic (Fink, 1919).

Rocha e Silva believes that desensitization might depend on an exhaustion of the mechanism leading to fibrinolysis and/or on the discharge of an antifibrinolytic agent that would block fibrinolysis.

(b) "Histamine Liberators"

There is actually very little difference between the manifest actions of peptone and other "histamine liberators" when injected into various species of animals. The distinction has been made in the present chapter mainly because the chemical nature of peptone is rather indefinite and also because peptone was the first of a great number of substances that have been shown to cause "anaphylactoid" shock.

When it was found that substances with better recognized chemical structures could be used in place of peptone for investigations on the release of histamine, the value of peptone in these studies declined. Much of our knowledge of the nature of histamine release can be attributed to the properties of these newer agents whose simple structures have guaranteed uniform and reproducible results.

The nature of "histamine liberators" has been extensively examined over the last few years and a consideration of these investigations follows.

(1) CURARE

Alam, et al (1939) found that curare released good amount of histamine when it was perfused through dog skeletal muscle or when injected in the whole animal. Schild and Gregory (1947), found similar results for tubocurarine. A recent report (Reid, 1950) indicated that the pattern of output by tubocurarine in dogs and guinea pigs was distinctly different from that caused by snake venoms and bacterial toxins and resembled more that seen in anaphylactic and peptone shock. As with peptone, tubocurarine

required the natural medium of blood for a maximal release of histamine. With Tyrodes solution or heparinized blood as the perfusion fluid only a small fraction of the liver histamine was releesed. In intact animals, after several injections of tubocurarine, the liver became insensitive to further injections after having released up to 50% of its content of histamine.

Rocha e Silva and Schild (1949) found that the liberation of histamine by tubocurarine was of an "explosive" type and was only limited by the rate of diffusion of histamine. The amount of histamine released depended largely on the concentration of curare used. In the perfused hind limbs of rats 20 molecules of tubocurarine were found necessary to release one molecule of histamine.

Collier and Macauley (1952) have recently shown that the long acting curarizing agents laudolissin and compound 15 (both chemically resembling dimethyltubocurarine) are both potent histamine liberators. Their investigations were done in cats (blood pressure), rats (suspended diaphragm), guinea pig (pithed) and man (skin tests).

(2) Basic Compounds

Much of the characterization of drugs as "histamine liberators" was done by MacIntosh and Paton (1949) whose classical paper outlined the basis for most subsequent investigations on the subject. Their experiments were done mostly in cats and dogs on a long series of compounds among which were included diamines, diamidines, diguanidines, diisothioureas, diquaternaries, some benzamidine derivatives and the polypeptide licheniformin. (See appendix for complete list of histamine liberators).

MacIntosh and Paton noticed that after the injection of the liberator, there was no immediate effect on the blood pressure. After the characteristic delay of 20-25 seconds the usually abrupt fall began; but its depth and duration depended on the dose of liberator. This delayed effect was attributed to the nature and location of the vessels into which the liberated histamine diffused. In the dog, the liver was the main site of histamine release; and in the cat, the skin and muscle were the main sites. The incoagulability of the blood in the dog after the injection of certain typical liberators appeared to be due to the release of heparin from the liver.

In this study particular attention was drawn to the fact that a large number of medicinally used organic substances, such as the trypanocidal diamidines, were capable of releasing histamine. In order to avoid such distressing side-effects as itching, colic and fall in blood pressure, such agents should be tested on animals for their histamine-releasing effects before they receive clinical trial.

MacIntosh and Paton suggested but rejected the possibility that a straight displacement mechanism operated to cause the release of histamine. The concomitant release of heparin in the dog could not be explained by this action because heparin is not a base, as is histamine, nor is it liberated by histamine. They presented no evidence to show that the mobilization of heparin depended on histamine release. As an alternative mechanism they suggested that histamine liberators act similarly to peptone by unmasking tissue or plasma proteases.

In this paper it was considered important that the action of the organic bases termed "histamine liberators" reproduces all of the manifest features of anaphylactic and peptone shock. It was also made clear by

their investigations that the degree of this "histamine liberator shock" can be controlled by adjusting the dose of the liberator. The idea of controlling anaphylaxis was previously unheard of because no two animals of the same species ever responded to the antigen to the same degree. With the advent of these new agents "controlled", "anaphylactoid" experiments became possible and would be very practical for the study of histamine release.

(3) Opium alkaloids

Feldberg and Paton (1951) continued with the project started by MacIntosh and Paton and introduced two new aspects of the subject. They showed that a number of opium alkaloids belonged to this class of chemicals called "histamine liberators" and they also identified certain tissues from which the histamine was released. Actually the work with opium alkaloids as releasers had begun many years before when Sollman and Pilcher (1917) had considered but rejected the possibility that these substances were able to release histamine. Lewis (1927) produced more substantial evidence by eliciting the triple response with very small doses of morphine. The next two investigations on opium alkaloids were reported simultaneously as short communications by Nasmyth and Stewart (1949) and Feldberg and Paton (1949). Both teams had found evidence that such alkaloids release histamine.

In their studies, Feldberg and Paton (1951) demonstrated that the depressor action of morphine on a cat's blood pressure was only partly due to the appearance of histamine in the plasma. The remainder was apparently due to the opiate itself. After the injection of histamine liberators into the perfused skin preparation of a cat histamine appeared in the effluent. With repeated injections almost all the skin histamine could be removed. The vasoconstriction and oedema associated with this

preparation were believed to be due to released histamine.

In perfused muscle preparations the amount of histamine released, even with repeated injections, represented only a minute fraction of the total amount.

Of special significance was the opinion of the writers that the release of histamine occurred explosively and that it appeared in the effluent only as it was washed out by the perfusing fluid. The mechanism of release was not discussed.

As in the earlier paper by MacIntosh and Paton (1949) these authors also suggested that some of the allergic phenomena associated with these compounds were probably due to their histamine-releasing action.

(4) 48/80

The histamine-releasing action of compound 48/80, a condensation product of p-methoxyphenethyl-methylamine with formaldehyde, was first demonstrated by Feldberg and Paton (1951). Its potency was apparently several hundred times that of tubocurarine, and it was calculated that 70-90 molecules of histamine were released per molecule of 48/80. Earlier, MacIntosh and Paton (1949) had calculated that diamidino-decane, then one of the most powerful releasers, required between 10 and 100 molecules to liberate one histamine molecule.

Investigations on dogs with 48/80 by Paton (1951) demonstrated a number of "new" pharmacological effects for "histamine liberators". As with other liberators, the principal actions of 48/80 resided in its remarkably high activity as a releaser; it apparently had no other pharmacological action. Paton showed, however, that it had the important property of releasing a "slow-contracting-substance". Beraldo (1950) had already shown the presence of such a substance in the blood of dogs exposed to

anaphylactic and peptone shock. Now, the discovery that releasers also mobilized this new entity further strengthened the belief that anaphylactic shock, peptone shock and liberator shock were mediated by the same mechanism. It was also shown that 48/80 possessed the ability to antagonize the guinea pig gut contraction specifically caused by the "slow-contracting-substance" even though it was itself inactive on the gut. This close relationship was considered by Paton to suggest that "slow-contracting-substance" was itself involved in the process of histamine release.

In species other than the dog a similar "slow-reacting substance" is also liberated in both anaphylactic and anaphylactoid shock (Feldberg and Kellaway, 1938; Brocklehurst, 1953). Since evidence is accumulating that these two conditions are alike in almost every detail, it is attractive to suppose that these obscure "slow-acting substances" are probably identical also.

Paton and Schachter (1951) have used gastric secretion in the dog as an index of histamine release caused by 48/80. Their experiments were designed to test the influence of antihistaminics on histamine release and resembled those of Grossman and Robertson (1948), who used benadryl to antagonize the releasing effects of Tween 20. Whereas Grossman and Robertson observed a great reduction in gastric secretion following benadryl, Paton and Schachter did not observe this effect after the antihistamine mepyramine. They asserted that the difference was due to the techniques used and that the gastric preparation and materials (especially benadryl) used by Grossman and Robertson were not specific enough to give accurate results.

Of special interest in this work with 48/80 was the fact that mepyramine eliminated many of the signs associated with histamine release, such as muzzle swelling and itchiness, even though the amount of gastric secretion was not reduced. Both of these investigations seemed to have ignored the fact (available at the time) that anti-histaminic drugs such as benadryl and mepyramine are probably themselves histamine-releasing agents (Pellerat and Murat, 1946; Arunlakshana, 1951). Because of this, even though the antihistamine may reduce the manifest action of released histamine on some parts of the animal's body, an accurate assessment of the part played in histamine release by compounds like 48/80 cannot be made on the basis of experiments with an antihistamine.

The repeated administration of 48/80, in Paton and Schachter's experiments (1951), was found to produce a temporary state of complete refractoriness to the same dose of the drug. This state, which almost disappeared after a week's time, was probably due to a depletion of the tissue histamine readily available to that dose of the drug. In this respect the refractory effects caused by doses of 48/80 seem to be very similar to those caused by the anaphylactic reaction.

Feldberg and Talesnik (1952) found that the rate of recovery of skin histamine in rats and dogs given subcutaneous injections of 48/80 was a remarkably slow process. In rats, the skin level returned to its normal level of 34 μg/gm. after a period of 30 days. A similar slow rate of recovery was noted in the dog. This slow recovery suggested:

(a) that the restoration of histamine was not into the depleted cells but was a sign of newly formed cells, and

(b) that the skin histamine played no role in purely physiological regulations, vascular or other.

If it is assumed that histamine is the first defense mechanism of the skin against injury, this could mean that once a cell has lost its histamine, it is unable to participate again in this mechanism and is therefore defenceless against further injury. If such is the case, then certain protective tissue cells probably die after loss of their tissue histamine. In a later paper, Feldberg and Talesnik (1953) refute this view because of new evidence that restoration also occurred in histamine-depleted striated muscleswhich regained their normal histamine store in about the same time as the skin.

Conversely, there is evidence to show that when "mast" cells age and die, a release of histamine and heparin takes place. Paff and Bloom (1949) believed that the liberation of heparin involved degenerative changes and even death of the mast cell. The high correlation in various tissues of histamine and heparin content with the number of mast cells (Riley and West, 1952) indicates that histamine release is probably also involved in this degenerative process.

The work of Riley and West (1952) has a bearing on another important aspect on the subject of histamine release. By using the fluorescent liberators, stilbamidine and 2-hydroxy stilbamidine they were able to observe that some of the liberator was temporarily trapped within the mast cell before release occurred. It is disappointing to find that their observations were not published in a more complete form as in this one short note subsequent histories of the liberator or mast cell were not given. Later work has suggested to these workers that the release of histamine in rat tissues is accompanied by a proportionate destruction of mast cells (Riley and West, 1953).

The effects of the chemical releasers 48/80 and d-tubocurarine, have recently been compared with the action of the specific antigen on

sensitized guinea pig tissues (Mongar and Schild, 1952). The nature of the release was apparently similar because the fraction of histamine liberated from 13 tissues was almost identical. (3% to 43% according to the tissue used). For all procedures there was no correlation between histamine content and fraction released.

In interaction experiments between 48/80 and the antigen Mongar (1952) and Schild found that whereas 48/80 greatly potentiated the anaphylactic reaction, the reverse was not true. They believed that each type of releaser probably caused a loosening of the bonds between histamine and non diffusible molecules in the skin to which histamine was attached. The firmness of the attaching bonds would then predetermine the amount of histamine released. Mongar and Schild do not suggest how the releasing action takes place other than that it involves loosening of certain bonds. The probability that other substances are involved in this action is discussed at the end of this chapter.

(5) MONOAMINES

Mongar and Schild (1953) have introduced the use of minced guinea pig lung as a means of comparing histamine-releasing ability of different substances. With this method a series of monoalkylamines, $C_n H_{2n+1} NH_2 HCl$ up to C 18 was tested and C 10 was 14 times more powerful than C_2 and 7 times moreso than 48/80 (!). On the other hand McIntyre, et al, (McIntyre, Roth and Sproull, 1951) had found that C_{18} was much more active than C_{12} in releasing histamine from rabbit's blood.

With the dibasic compounds it had seemed reasonable to assume that their activity relied on goodness of fit of the two basic groups to bifunctional receptors in the tissue. The same explanation seems inapplicable to the results of Mongar and Schild. They suggest that

maximum activity at C_{10} is probably the result of two opposing processes: increasing activity with increasing chain length and decreasing activity with the increasing tendency to form micelles (aggregates).

(6) DIAMINES

Mongar and Schild (1953) have recently investigated the effects of a number of simple diamines of the homologous series $NH_2 (CH_2)_n NH_2 - 2HCl$. They found that the activity of the diamines as histamine potentiators paralleled their activity as histaminase inhibitors with the maximum of both at n=5. Potentiation was evidently produced by combination with the histamine receptors on the enzyme. The histamine releasing action of the diamines paralleled their antihistamine action on the guinea pig gut. This latter was a non-specific action as the response to acetyl-choline was similarly depressed. Both release and depression were more marked as the chain length increased up to n=15. With regard to their earlier investigations the mode of action of monoamines as liberators was thought to be the same as diamines.

(7) Miscellaneous compounds
Adrenaline

The range of compounds classified as "releasers" has extended to as important a group of chemicals as the sympathicomimetics. In 1946, Staub reported an increased plasma histamine level during intravenous infusions of adrenaline in man (20µg per minute) and suggested that some of the cardiovascular effects of adrenalin may be explained on the basis of histamine release. Other workers (Eichler and Barfuss, 1940; Koch, Szerb, 1950) have also reported the release of histamine from tissues by adrenaline.

Whelan (1952) and Mongar and Whelan (1952) reported that they were unable to find an increase in the blood histamine level of the human forearm circulation after infusions of adrenaline over a wide range of doses and therefore concluded that the vasodilator effect of adrenaline could not be explained by the histamine released. Vasodilator effects were obtained with doses of adrenaline as small as 0.001 μg/min. but no histamine was detected when doses of adrenalin up to 20,000 times this value were given. An "after-dilatation" occurring subsequent to the adrenaline perfusion in denevated forearms also could not be explained by the authors on the basis of a histamine mechanism. The release of histamine subsequent to increased cardiac activity caused by adrenaline has been discussed in an earlier section.

Pseudoallergens

It had long been known that certain compounds regularly evoked drugs an "allergic" response in many subjects. A comparison of the symptoms exhibited by dogs receiving these compounds to that of primary histamine releasers such as 48/80, suggested to Schachter (1952) a similar mode of action. Schachter conducted experiments on intact cats and also on skin preparations of dogs and cats to test his hypothesis. Drugs found to release histamine were pethidine, atropine, quinine, priscol, neoarsphenamine, and bile salt. Substances like sodium bromide, hydrochloric acid, distilled water and saline were without effect as was anoxia. Sulphanilamide and dinitrophenol also failed to release histamine but it is interesting to note that second administrations of these compounds usually cause atopic reactions. (Boyd, 1947).

Polypeptides

The only structural prerequisite for histamine releasing ability

so far entertained has been that mentioned by MacIntosh and Paton (1949). Thus many releasers have been shown to possess a chemical structure of the general formula R.X.R' where R. is a basic group and R' is basic as in diamino decane or polar as in adrenaline. X is an inert moiety of mainly hydrocarbon nature. Among the natural releasers having this chemistry are polypeptides such as peptones and licheniformin that contain a high proportion of basic amino acids. Snake and bee venoms are thought to act, partly at least, by the formation of peptones produced by proteolysis.

Among "newer" histamine releasers that may perhaps have the proposed structure are natural proteins such as are contained in horse serum and egg albumen. Feldberg and Schachter (1952) were able to demonstrate that horse serum released histamine in the non-sensitized cat. Experiments were performed on cat and dog skin preparations and comparisons were made using 48/80. In the dog only sensitized preparations released histamine when the specific antigen was added to the perfusion fluid. In contrast to results with 48/80, the release was delayed and protracted, and the preparation also could not be depleted with repeated injections of horse serum.

In the skin preparation of non-sensitized cats, horse serum (but not the sera of dogs, rabbits or cats) caused the release of histamine. Boiled horse serum not only failed to release histamine but also prevented the release by normal serum, although the response to 48/80 was unaffected. The release by horse serum in cats resembled that of 48/80, being of a similar explosive nature.

Using egg white, Schachter and Talesnik (1952) performed similar experiments and were able to demonstrate histamine release in non-

sensitized cats and rats but not in the dog. Although the syndromes were all produced by the ovomucoid protein fraction of egg white, the histamine releasing constituents were found to be different for the rat and cat, since in the former it was a heat labile and in the latter a heat stable component.

In 1937, Menkin isolated from human exudate a substance with chemotactic properties and the ability to increase capillary permeability. This substance, called "leucotaxine" was believed by Duthie and Chain (1939) to be composed of a number of polypeptides. They also supposed that such a substance must be "largely responsible for the permeability in capillaries which always occurs in the later stages of inflammation after proteolysis in the body has begun".

In 1949 Dekanski (1949) investigated farther the nature of leucotaxines action. He found evidence that leucotaxines' effect in the trypan-blue permeability test in the cats' skin was due to its ability to increase the histamine content of the skin. Histamine then mediated the escape of the dye from the circulation. Dekanski compared the blueing to that seen with peptone, heat and histamine and suggested that the effect was due to "histamine formation".

In 1952, the problem of capillary permeability mediation by histamine was investigated by Miles and Miles (1952). They used guinea pigs injected with the due Pontamine Sky-blue 6X and compared the skin effects of histamine, leucotaxine and 48/80. The similar results for 48/80 and leucotaxine suggested they act by means of the same mechanism. All three substances increased capillary permeability and also induced a cross-immunity to one another. This last effect is apparently typical of all histamine releasing agents (Lewis and Grant, 1924).

Miles and Miles pointed out that much of the histamine available for release was located in vascular regions. This fact, together with the knowledge that heparin is also located in these areas, in the mast cells (Jorpes, 1946), confirms the belief that histamine and heparin probably have a common cellular origin (Riley and West, 1952).

Rubini, Becker, and Stahmann (1953) have investigated some of the pharmacological effects of lysine polypeptides. Although no investigation of histamine liberation was made, some of their data suggest that such compounds as these would be capable of producing that effect.

Because of the fact that several protein products and organic bases produce anaphylactoid shock in various animals, Jaques (1949) attempted to show that the protamine salmine had a similar effect in dogs. When doses up to 10 mg/kg. were given no histamine could be detected in the blood. However many of the symptoms of anaphylactoid shock occurred, and some of the outward signs could be decreased by the antihistaminic Benadryl. In these experiments probably clearer results could have been obtained if larger doses of the protamine had been given. The reason for this belief will be made clear in a later section.

TWEEN

Detergents of the Tween series have been shown to possess potent histamine liberating ability (Krantz, Carr, Bird and Cook, 1948; Krantz, Carr, Bubert and Bird, 1949; Grossman and Robertson, 1948). These substance does not have the structure typical of histamine liberators and their the mode of its action is not known. Chemically, Tween 20 is a polyoxyethylene derivative of sorbitan monolaureate.

Goth, Allman, Merrit, and Holman (1951) have found that the release of histamine by Tween 20 causes a refractoriness in dogs, probably the same as that caused by other liberators. This refractoriness was prolonged even more when the dog received cortisone. The nature of this prolongation was explained by them as probably being due to the prevention of the replenishing of depleted tissue histamine stores. In the light of Ungar's recent work (Ungar and Damgaard, 1951.) the action of cortisone is explained on the basis of an increased action of tissue antifibrinolysin.

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In the above chapter the effects of histamine liberators in various animals and animal tissues have been shown. The obvious similarity between the manifest effects caused by liberators and those seen in anaphylactic shock suggests that the mechanism underlying the two reactions are very much the same. The apparent similarity between the reactions of anaphylactic shock and those produced by such compounds as peptone had led earlier investigators to term the latter condition "anaphylactoid" shock. Later the symptoms of these two types of shock were shown to be largely attributable to histamine released in the reaction.

Early "histamine liberators" were given that name because their most obvious action was to cause the release of histamine. Subsequent studies, however, have shown that the total effect following the injection of histamine liberators into animals is the same as that shown for both anaphylactic and anaphylactoid shock. It is very probable that "histamine liberators" could be very well classed with those compounds whose action had led to the term "anaphylactoid shock".

However, as investigations are still in progress to elucidate the nature of these phenomena, this identification has not been made. The most appropriate designation for these substances ("histamine liberators") therefore seems to be "anaphylacto-mimetic agents". In spite of this suggestion the author has used the term "histamine liberator" throughout the thesis.

In this section it has become apparent that many useful drugs such as curare and neoantergan have had an undesirable side action, the property of histamine release: and it will be seen in the appendix that the number of drugs having this property is already considerable. The list will undoubtedly increase further with the synthesis of new drugs, many of which are chemically closely related to histamine releasers. It will therefore become increasingly important to determine the histamine-releasing potency of such compounds before they are made available for clinical or public use. For important drugs already in use, prophylactic measures against their histamine-releasing side effect should also be instituted as soon as possible. In the experimental section a study of attempted prophylactic procedures has been recorded.

6. SUMMARY OF THE INTRODUCTION

In this chapter an attempt has been made to show the various techniques by which the release of histamine takes place. Although a complete list of methods was not covered, it should not be very difficult for the reader to fit any other substance or mechanism into one of the categories mentioned.

As the subject is covered, one gradually comes upon the conviction that histamine release takes place almost entirely, if not entirely by the activation of enzymes. The only other mechanism that has been suggested to cause the release of histamine is the displacement hypothesis of MacIntosh and Paton (1949). However the authors themselves have rejected this possibility, as have Mongar and Schild (1953 b) who worked with similar compounds. Both teams of investigators seemed more inclined to believe that histamine release occurred as a result of enzyme participation in the reaction.

One of the stumbling blocks to further research seems to be the lack of knowledge of how enzyme activation occurs. Thus far only one prerequisite for the reaction has been suggested: that the proposed histamine releaser must have one or more amine molecules in its structure. The only known exceptions to this "rule" is for compounds of the Tween series (Goth, Allman, et al. 1951) and for the bile salts (Schachter, 1952), but no suggestion has been made to account for their specific action. MacIntosh and Paton (1949) were unable to find any correlation between the surface activity of a number of such substances and their depressor activity.

The wide variation in the structures of those compounds having amine groups has not permitted a very good correlation between structures and histamine releasing power. So far, the distance between the basic groups in straight chain diamines, diguanidines, diamidines etc., dibenzamidine groups or between a benzamidine and a second basic polar group has been found to have a relation to the magnitude of the reaction. A maximum histamine releasing ability was found in compounds of this kind having a chain length of about ten carbons. (MacIntosh and Paton (1949). In the case of monoalkylamines also, C_{10} appears to be the most

potent compound (Mongar and Schild, 1953 a). However the structures of longer chain polypeptides are not so easily correlated with their histamine releasing activity.

Histamine releasing agents form highly alkaline solutions when dissolved in water but it is not known if the pK values of these substances have any correlation with their potencies as liberators. Some of the compounds listed by MacIntosh and Paton (1949) would appear to cause a depressor effect in proportion to their basicities but no precise correlation was made.

Although the evidence for the release of histamine by enzymes is much clearer than that for other mechanisms there are some types of histamine release for which no mechanism has been conclusively demonstrated as yet.

One of the most puzzling instances is the question whether there is actually a release of histamine in functional metabolic activity. It has certainly never been adequately shown that histamine release is a participating factor in, or a result of, normal tissue activity. Practically all of the methods used to show this effect have had to exaggerate normal metabolic levels or else introduce new factors into the system. If it is supposed that histamine release only occurs after the threshold of a specific activity is reached, one is certainly not allowed to increase that activity, measure an increased level of histamine and then suggest that the release of histamine takes place as a normal tissue function.

The "flare" of the "triple response" which occurs so readily after the slightest injury is indicative of the delicacy of the mechanism that leads to the release of histamine. In the future it will probably be a simple matter to show whether or not histamine is released during

a procedure proposed to measure the release. The major difficulty will be to determine whether the histamine collected under the conditions of the experiment is formed normally or as a result of abnormal activation of the histamine releasing mechanism.

The activation of proteolytic enzymes in tissues and serum has been demonstrated by various techniques but there is little evidence to show that this is a normal metabolic occurrence. Ratnoff (1953) has shown that the clotting process activates a fibrinolytic enzyme but it seems doubtful that the action of thromboplastin is the mechanism that initiates the liberation of histamine to regulate local vascular changes. Possibly the local activation of the fibrinolytic system by other mechanisms could lead to the release of small amounts of histamine. It is a matter for speculation that this will eventually be demonstrated in living tissues.

The suggestion that placentation of the ovum takes place by the release of histamine has supplied better evidence that histamine has a part in natural metabolic activity. (Shelesnyak 1952). Perhaps the participation of histamine in the process of nidation is one of its true normal metabolic functions. However, this study has not added anything to our knowledge of how the release of histamine takes place.

Evidence for the participation of enzymes in the liberation of histamine by other stimuli is rather good. Many of the systems described have seemed to initiate their effect by various kinds of tissue damage and there is no doubt this itself probably releases varying amounts of histamine. (Trethewie 1938). However some of these investigations have also shown that activated proteases may themselves have caused proteolytic destruction of the tissue and thereby released

histamine.

The release of histamine by chloroform, as shown by the red "flare" response, is not apparently due to tissue damage but occurs as a result of mild irritation of the tissue cells. Other studies (Kaplan, 1944; Ungar and Mist, 1949) indicate that the chloroform activates a fibrinolysin and this leads to the release of histamine.

The two most intensely studied mechanisms causing histamine release are those of anaphylactic and anaphylactoid shock. Except for the sensitization period necessary to produce anaphylactic phenomena the two mechanisms seem to bring about the release of histamine in the same way. The experiments of Rocha e Silva and Grana (1946), Rocha e Silva, Scroggie, Fidler, Jaques (1947), Scroggie, Jaques and Rocha e Silva (1947) and Scroggie and Jaques (1949) on anaphylactic and peptone shock have shown that these mechanisms, in dogs, are parallel from the time of the injection of antigen or peptone to the release of histamine. As well as histamine, heparin and a "slow-reacting-substance" were also released. The white blood cell count falls sharply, enormous clumps of leucocytes and platelets suddenly disintegrate, and an activation of the plasma protease occurs.

In the section entitled "histamine liberators" other features common to the two systems were seen. In particular, the studies of Mongar and Schild (1952) leave little doubt that the reactions are almost identical. The fraction of histamine released by the two substances 48/80 and antigen, were found to be very much the same for either normal or sensitized tissues, but the concentration of histamine in any one tissue did not predetermine the fraction released. The explanation for the parallelism between histamine

released by chemical releasors and antigen suggested by Mongar and Schild was that some degradation product of proteins was formed by the antibody-antigen reaction and had peptone-like effects. However other experiments by these authors (same paper) suggested to them a rather more complex explanation. Since the action of the chemical releaser seemed to potentiate the antibody-antigen reaction but was not potentiated by it, the effect of the former was thought to be probably further reaching and more permanent. Each type of release was believed to involve a loosening of the bonds between histamine and non-diffusable molecules in the cell to which the histamine was attached. It is odd that enzyme systems were not mentioned as a possible mechanism of release. What the authors meant by "involved a loosening of the bonds" is not clear. They are not, apparently, referring to the action of the releasers themselves since these substances are suppliable at the experimenters wish, and if the compounds caused release directly, surely the histamine stores could be fully depleted with sufficient releaser. Since not even the largest concentrations of 48/80 and/or antigen, were able to release more than a certain percentage of histamine, it is doubtful that they themselves have a simple bond splitting action.

The authors apparently did not follow up their results with experiments to show if enzyme systems were involved in the mechanism of histamine release. The amount of "fibrinolysin" activated in each type of tissue by the two mechanisms would quickly show them if a correlation existed. It is suggested that a high correlation probably would exist between the degree of protease activation and the percentage of histamine release. To be more precise, it is further suggested that the amount of histamine release may be a function of the "fibrinolysin" available. (In this paragraph "fibrinolysin" has been used to refer to the activated tissue enzyme.)

Investigations along this line have recently been done by Ungar Damgaard, and Hummel. (1953). It is well known that profibrinolysin (P.F.L.) activation occurs when the specific antigen is added to tissue suspensions or serum sensitized animals (Ungar, 1947; Ungar and Mist, 1949; Geiger, 1952). Ungar reports that P.F.L. activation also takes place when histamine liberators such as 48/80 or DA10 are mixed with serum or tissue suspensions and that a complete parallelism exists between conditions under which P.F.L. is activated and histamine is released. Samples of guinea pig tissue suspension fluid were collected at varying intervals, and tested for proteolysis and histamine. Histamine release was found to occur whenever proteolysis reached a certain initial rate. The ratio between release and proteolysis remained constant during the first 10 minute period. F.L., used at concentrations similar to those obtained by P.F.L. activation, also caused the release of histamine. Streptokinase produced the same effect.

Ungar remarks that these results support the view that during anaphylactic and anaphylactoid reactions the following sequence of events take place.

P.F.L. Activation ----------> F.L. in active form

---> Breakdown of proteins to which histamine is bound.

---> Release of free histamine

The amount of histamine in a readily available form has been suggested (Feldberg and Talesnik, 1953) to be correlated with the susceptibility of the histamine of various tissues to 48/80. Rats which had received one dose of 48/80 were found to be refractory to subsequent doses in that they did not display the usual signs. This

was thought to be due to a previous high loss of body histamine. As these experiments were done in whole animals, and only skin histamine estimations were made, it is not known to what degree other factors, such as enzyme, took place in the reaction. They had found that 90% of skin histamine was lost after the intraperitoneal injection of 0.5 mg. of 48/80. Mongar and Schild (1952) had found that the isolated skin of guinea pigs released only 30% of their histamine. Is there a reason besides species difference, for this disparity? It is suggested that in the intact rat large quantities of fibrinolysin may have been activated and made available for the release of histamine throughout the body. Thus in a live animal, refractoriness of histamine releasers may well be due to the loss of large amounts of histamine in all parts of the body. However, refractoriness in isolated ("still living?") tissues is probably more due to the loss of factors mediating the release of histamine. Mongar and Schild (1952) showed that if sufficient time was allowed between injections of 48/80 given to contract an isolated guinea pig uterus, the effect could be repeated without tachyphylaxis. They did not speculate on why this was so but there exist two possible reasons. The (living) uterus had either produced (a) more histamine accessible for release or (b) more of the factors necessary for the release of histamine.

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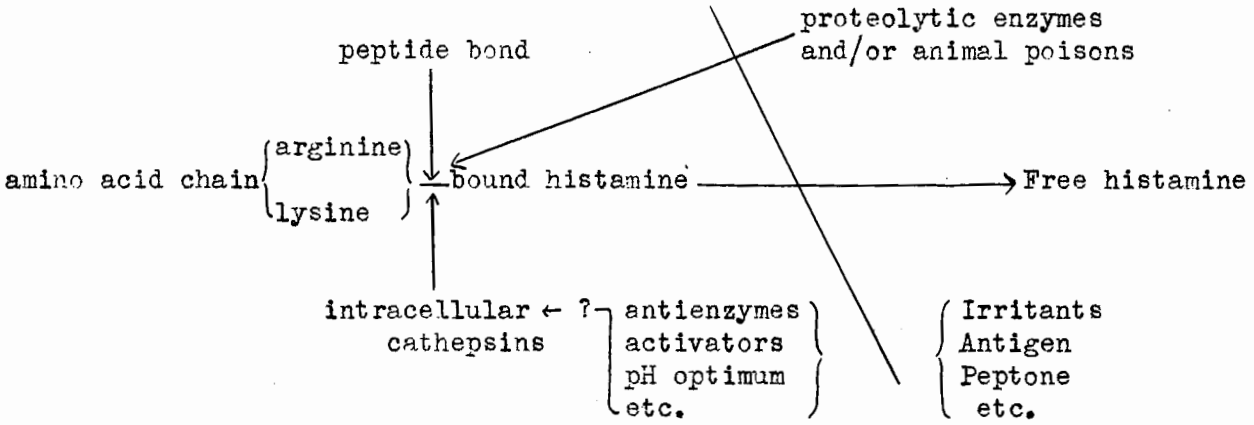
Feldberg (1941) believes that histamine might be released from a lipoprotein complex by the action of any hemolytic agent capable of splitting lipoid substances of a lecithin-like character. Rocha e Silva (1944) believes that histamine is released by the action of enzymes at the peptide bond which holds histamine to an amino acid

chain. He suggests that this bond may be split by one of two ways. Either a native intracellular cathepsin is activated to split the bond by such substances as peptone, antigen, or irritants; or the bond is attacked by foreign enzymes such as are in toxins or venoms. The mechanisms of this scheme is shown in the following (Rocha e Silva, 1944).



The question mark indicates that is is not yet known how the native enzyme system is activated.

The "native" enzyme system, however, is apparantly not entirely found within the cell from which histamine is released. Such factors as F.L. and P.F.L. have all been isolated from the plasma. The studies of Rocha e Silva (1950) indicate that these plasma factors are especially important for the release of histamine in the dog.

In the section on enzymes it was shown that several agents and techniques are available for the activation of the serum protease usually designated by fibrinolysin. (Ferguson 1948). It has occurred to the author that many of these features could be combined in a single scheme. The basis for the proposed scheme was obtained from a paper by Seegers (1950) who has considered the blood clotting system as being composed of two basic mechanisms: one is involved with coagulation,

the other with lysis. However, the two are not opposed to each other and each has an important metabolic function. Primarily, the function of the lytic system can be regarded as having to do with the fibrin clot but this does not forbid its participation in other systems.

The following diagram (Figure 2) is basically that of Seegers (1950) but a number of factors which probably take part in the process, especially in relation to the fibrinolytic system, have been added to clarify (?) the picture. It is hoped that the large number of activating factors listed as profibrinolysin activators will not further confuse the reader since only one factor (11) is shown to take part in the A.F.L. - F.L. separation. As far as is known none of the factors listed have been found incapable of transforming P.F.L. to F.L.

The following are the references for Figure 2 (next page).

(1) Ungar and Damgaard (1951).

(2) Ratnoff (1953).

(3) Antifibrinolysin provided by platelets; Johnson and Schneider (1953). Antifibrinolysin decreased with injury at first, then increased (see 7)

(4) Lewis and Ferguson (1951).

(5) Ferguson, Travis and Gerheim (1952).

(6) Lundblad, (1950).

(7) Cliffton (1952).

(8) Permin (1950).

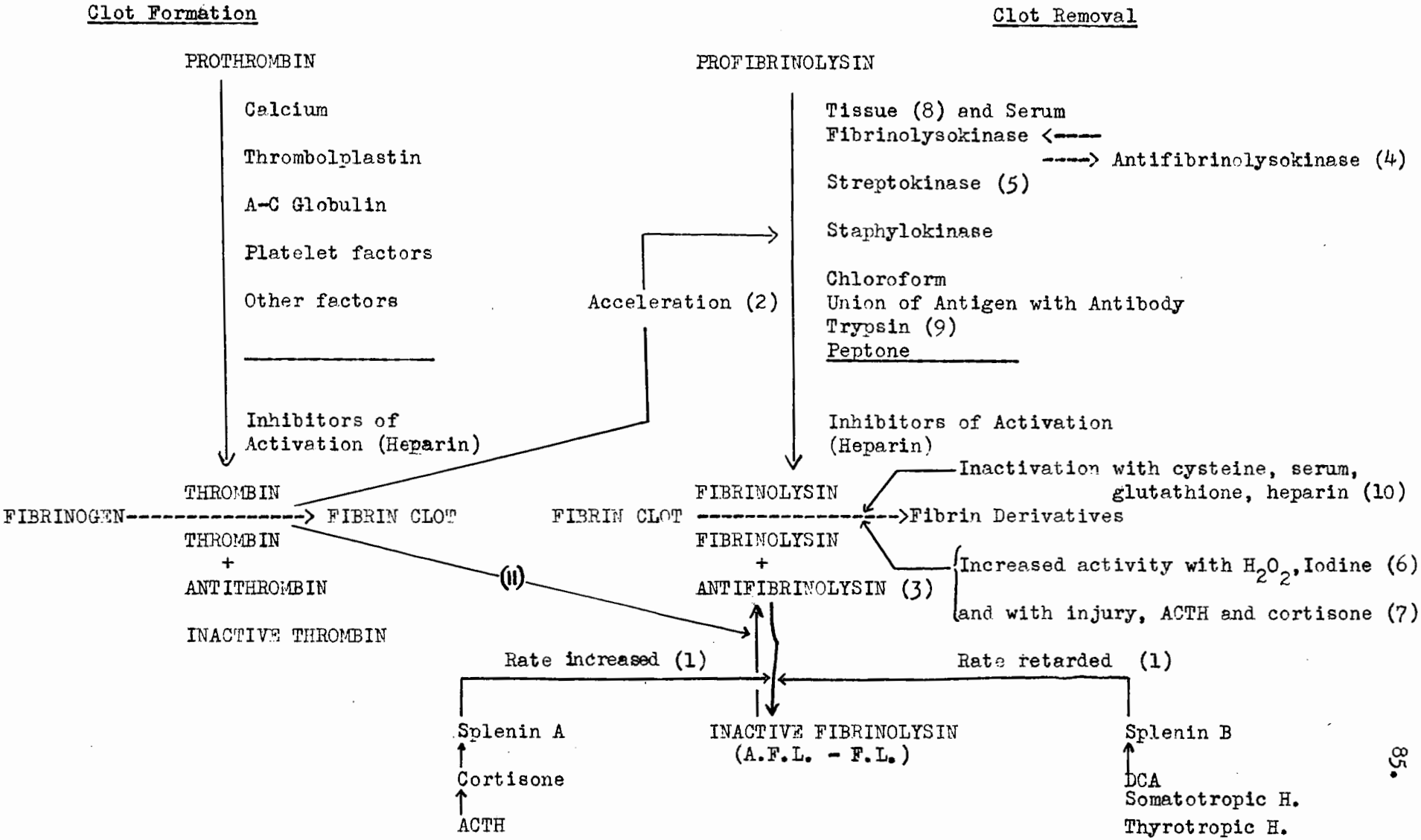
(9) Astrup and Sterndorff, (1950).

(10) Astrup, Crookston and MacIntyre (1950).

(11) Schmitz (1937).

Figure 2

THE CLOTTING OF BLOOD (Seegers 1950)



A possible mechanism participating in the release of histamine which has not yet been discussed in the thesis may eventually prove very important. Blaschko and Hawkins (1950) found that the affinity for the alkane diamines of histaminase and amine oxidase caused the diamines to be broken down very rapidly. However, the maximum activity for the enzymes histaminase and amine oxidase was found to be respectively against the C_4 and C_{13} diamines. MacIntosh and Paton (1949) and Alles, et al. (1953) had found that the C_{10} diamine had the greatest amount of histamine releasing activity, and the results of Blaschko and Hawkins (1950) indicate that the diamines of this length have more affinity for amine oxidase than for histaminase. Therefore it seems possible that the inhibition of amine oxidase may have some importance in the release of histamine. This aspect of the problem has not been dealt with in the experimental work but it is suggested that experiments with amine oxidase inhibitors, such as ephedrine, may help to elucidate the part played by amine oxidase in the release of histamine.

PART 2. EXPERIMENTAL WORK

The body of work deals with procedures and methods, including the administration of chemical compounds, concerned with the release of histamine in animals. Routine procedures will be described in this section. Methods used in special experiments will be described with the experiment under consideration.

A. ROUTINE PROCEDURES

1. The Extraction of Histamine

The method used for extraction of histamine from skin was essentially that of Best and McHenry (1930) and from liver that of Barsoum and Gaddum (1935). The extractions were carried out as follows:

(a) Skin

The freshly removed skin sample which had been clipped free of hair was rinsed quickly in saline and then gently pressed between filter papers to remove excess moisture. The tissue, usually less than one gram, was weighed in tared 100ml. beakers. 3ml. of conc. HCl and 7 ml. of water were then added to the beaker which was placed in a water bath at 98°C for 1 hour. Excess evaporation was prevented by covering the beakers with watch glasses. At the end of the hour the clear solutions were neutralized with 10N NaOH and diluted to isotonicity with distilled water. Further dilutions were made with 0.85% saline. The samples could be assayed immediately or stored in a refrigerator.

(b) Liver

Freshly removed liver samples were finely minced with scissors and placed in a 10% solution of trichloracetic acid, 5ml. per gram of

tissue. 0.5 ml. of conc. HCl per gram of tissue was added to this and the whole allowed to stand for 16-20 hours. It was then filtered through a Buchner funnel, carefully washing the precipitate with 1/5 volume of trichloracetic acid. The clear filtrate was transferred to beakers and heated in a water bath for 1/2 hour at 98°C. Excess evaporation was prevented by the use of watch glasses. The solution was neutralized to pH 7.5 with 10 N NaOH and diluted to isotonicity with distilled water. Further dilutions were made with saline or the solutions were stored in the refrigerator.

(c) Blood

Blood plasma samples were usually not treated prior to the assay for histamine. 10 ml. samples of blood were taken and 1 ml. of 1.0% heparin used as the anticoagulant. If heparin estimations were to be made (metachromatic assay) 1 ml. of 3.8% sodium citrate was employed as the anti-clotting agent. In some experiments where difficulties were being encountered in histamine assays using the guinea pig preparation, the method of Code (1937) was used to obviate the presence in the samples of interfering factors. 20 mls. of blood were collected in 1/20 volume of 1% heparin, spun at 1500 r.p.m. for 20 minutes. The plasma was then mixed with 15 mls. of trichloracetic acid. After standing for one hour this was filtered and the precipitate washed with 4-5 ml. portions of the acid. 10 mls. of conc. HCl was then added to the filtrate and the mixture was boiled for 90 minutes over a low flame. A watch glass over the beaker prevented undue evaporation. The mixture was then neutralized with NaOH and diluted to isotonicity. Extracts prepared in this way were assayed for histamine on the gut without difficulty.

Blood samples were always taken from the femoral artery using a siliconed steel cannula. After each sample the cannula was rinsed with 10 ml. of saline and then filled with the anticoagulant. This was removed before the next sample was taken. The first ml. or so of blood from the cannula was rejected before the collection was made. Immediately after taking, the sample was spun at 1500 r.p.m. for 15 minutes. The plasma was then removed with a pipette and placed in clean tubes.

2. Methods of Histamine Assay

(a) Guinea Pig Intestine Preparation

This preparation was first described by Guggenheim and Loeffler in 1916. Since that time its value as a test object for histamine has not been equalled, either in sensitivity or in accuracy, by other assay techniques.

(1) The Apparatus (Figure 3)

A plastic 12x7x7 inch constant temperature water bath was arranged for facilitation of the assay. The bath was set at 31°C and was found to stay at this temperature remarkably well. Heat was supplied by 4 resistance heaters placed under the plastic case.

The 5 ml. bath which contained the suspended gut preparation was supported by the glass outflow tube which led to a rubber draining tube. (See diagram). The inlet tube which entered the bath through the same bung as the outlet tube carried fresh Tyrode solution to the preparation. The temperature of the Tyrode was equalized to that of the bath by means of a coil placed in the larger bath. Spring clips regulated the flow of solution through the bath.

A hook on the oxygen inlet, which was suspended above the bath, provided a means for attaching the gut within the bath. One thread

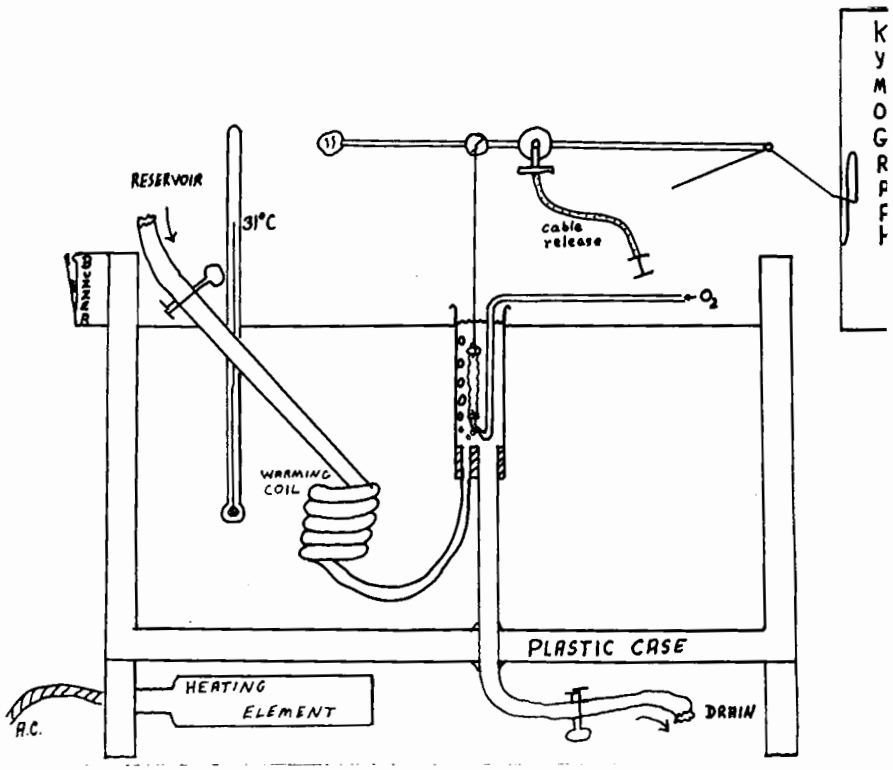


Figure 3.

Apparatus employed in the assay of histamine utilizing the guinea pig ileum preparation. The gut is suspended in a 5 ml. bath by cotton threads between the writing lever and a hook on the glass oxygen inlet. Fresh Tyrode solution is run into the bath after each contraction of the gut caused by the addition to the bath of histamine or other pharmacologically active substances.

attached the gut to this hook and another thread fixed the upper end of the gut to the writing lever located directly above it.

The thread was attached to the lever by means of a small piece of plasticine which also served to balance the lever. Another piece of plasticine was at the end of this part of the lever. The lever itself was equipped with a frontal writing point which produced almost perpendicular records on the slowly moving (5 mm./min.) kymograph drum. A cable release at the fulcrum of the lever permitted steadying of the lever when the bath fluid was renewed.

To help eliminate any sticking of the stylus during the period of contraction a very small vibrator was attached to the side of the bath or to the arm which held the lever fulcrum.

The Tyrode solution was made up fresh usually a few hours before each assay. The formula was as follows:

NaCl	9.00 grams	$MgCl_2$	0.005 grams
KCl	0.42 grams	$NaHCO_3$	0.50 grams
$CaCl_2$	0.24 grams	Glycose	1.0 grams

Glass distilled water to 1000 ml.

The mixing procedure was found to be important as clouding of the solution occurred if the bicarbonate was added directly to the solution containing the other salts. Therefore the bicarbonate solution was made up in a separate flask and then the two solutions were mixed.

(2) Preparation of the Gut.

Guinea pigs of either sex weighing 200 - 300 grams were killed by a blow on the head followed by decapitation. The abdomen was then opened and a piece of terminal ileum 20-30 cms. long was removed. This strip was then subdivided into smaller 8-10 cm. lengths and

gently washed through with Tyrode solution. All portions touched or handled in the procedure were rejected. Pieces that were not used immediately were stored in the refrigerator but they did not seem to have any particular advantage over sections not so stored.

Sections about 2 1/2 cm. long having a wrinkled or dimpled appearange were chosen for the assay. Threads were passed through both ends with a sharp three-edged needle and then tied so that the gut remained open at each end. This procedure was facilitated by using a petri dish to keep the gut wet while it was being attached to the oxygen hook. The hook with the gut attached was then transferred to the bath and adjusted for maximum efficiency. The oxygen was turned on to form a continuous stream of small bubbles in the bath.

After several washings the gut was usually ready for the test. If not, it could be "sensitized" by a large (5µg.) dose of histamine. Once established, the sensitivity of the preparation remained reasonably constant for 5 or more hours.

(3) Preparation of the Histamine Standard

Throughout the study the dihydrochloride of histamine was used. Therefore 100 mls. of a 10^{-3} solution equivalent to the histamine base was made up by dissolving 167 mgs. of the dihydrochloride in 100 mls. of glass distilled water. This solution remained stable for several months if kept in a refigerator. 10^{-5} or weaker solutions were made up freshly in saline from the 10^{-3} standard.

During the assay the strengths of either the standard or unknown sample could be adjusted so that between 0.1 and 0.2 ml. was injected into the 5 ml. bath. After each refilling of the bath, the regular flow of oxygen bubbles to the surface adjusted the bath level to a constant height about 1 mm. below the height of the glass bath. No

other precaution was felt necessary to assure a constant bath volume. For rough estimation the fluid level of the bath was lowered slightly to accomodate up to 0.5 ml. of the unknown.

(4) The Assay of Histamine

For 0.020 µg. of histamine the gut usually gave a response measuring 5 to 8 cms. on the kymograph record. The sensitivity of the preparation was such that differences as small as 0.001 or 0.002 µg. of histamine could be measured with accuracy. This is seen in Figure 4. During the assay small variations in the response sometimes occurred but as comparisons with the standard were being made continually this could be ignored. All injections were made with 1 ml. tuberculin type syringes equipped with 1 1/2" No. 20 needles.

Comparisons could usually be made every 1 1/2 minutes but sluggish pieces of gut sometimes needed a few more seconds. This was especially true if the samples contained a "slow-contracting substance". After every injection of unknown a comparison was made with the histamine standard.

Suspicions of sensitization of the gut by the samples tested could be tested by giving several unknowns and then several standard histamines. Another test of sensitization could be made by superimposing injections of histamine or unknown on the gut already contracted by one of these. With both these methods abnormal responses of the gut to the sample or to histamine would indicate the presence in them of sensitizing substances.

Antihistaminics and atropine were used routinely for the identification of histamine in plasma and tissue samples. Neoantergan maleate (= mepyramine maleate) at a concentration of 10^{-9} in the bath usually reduced by 2/3 or more the response to histamine. Neo-

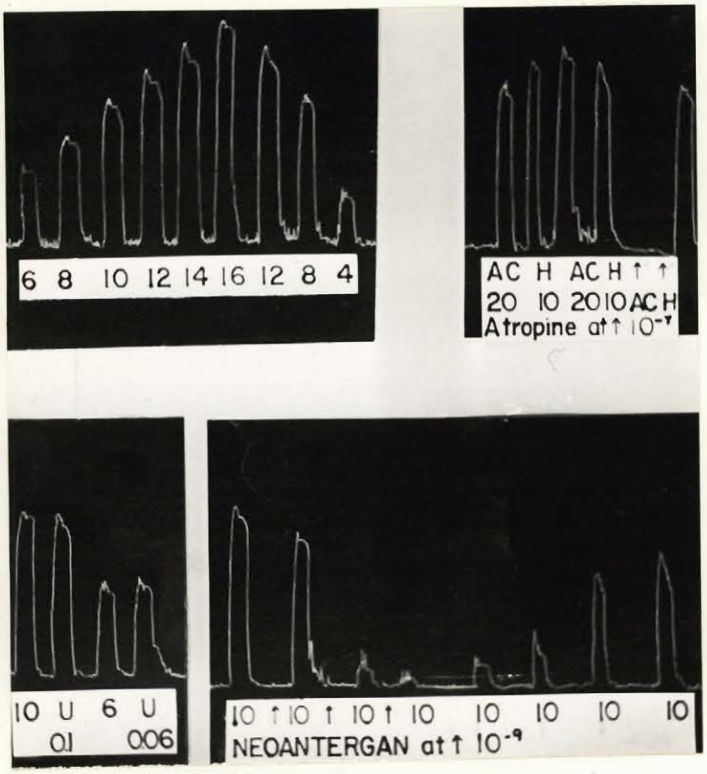


Figure 4.

Examples of recordings taken during histamine assays using the guinea pig ileum preparation. Figures refer to additions to the bath of histamine or acetylcholine in mμg.

Upper Left Record of responses by the gut caused by the addition to the bath of increasing and decreasing amounts of histamine. The additions were made two minutes apart. It is notable that the gut responds in a graded manner to amounts of histamine different by only 2 mμg. Sensitization of the gut to histamine has occurred at the right of this record.

Upper Right The effect of atropine on matching doses of histamine and acetylcholine is shown. At a concentration of 10^{-7} of atropine, the action of acetylcholine has been abolished but that of histamine has not been greatly affected.

Lower Left A solution containing an unknown (U) amount of histamine has been matched by known values of histamine at two levels. The unknown solution is thus verified to contain 100 mμg histamine /ml.

Lower Right The addition of Neoantergan to the bath has lowered contractions of the gut in response to histamine. In the absence of neoantergan the gut returns in a short time to normal sensitivity.

antergan was allowed to act in the bath 30 seconds before the histamine was added. When the antihistaminic reduced the effects of an unknown sample and of a matching dose of histamine to just the same degree, it was considered that the sample owed its activity to histamine. After this, with neoantergan omitted from the bath, equal responses of the gut to histamine and to an unknown containing histamine could be observed to return to normal at equal rates. See Figure 4 for an example of the use of neoantergan.

Atropine tests were carried out after equal responses to histamine and acetylcholine were established. 0.05 ml. of a 10^{-5} solution of atropine usually abolished the response to acetylcholine but had little effect on the histamine response. Unknown samples that contained only histamine also would not be affected. (Figure 4).

For all estimations where the plasma samples were known to contain drugs that might influence the response of the preparation to histamine, the effect of this substance on the gut was also determined.

(b) Cat Blood Pressure

This method was described by Burn in 1928.

Cats of either sex weighing 1.5 to 3.0 Kg. were used. Chloralose, 80 mg./Kg. in a 1% solution was used as the anesthetic and was given in one of two ways. By intraperitoneal injection the animal was usually ready in 30 minutes but the period of induction was sometimes accompanied by vomiting and defecation. If ether was used initially the period of induction was shorter and more pleasant, and the chloralose could be administered intravenously as soon as a vein was exposed. Using the latter technique we have had very few difficulties and have generally found that the preparation was a more satisfactory one.

All injections were made through the same glass cannula as was used for administering the chloralose and the solution was washed into the animal with saline which flowed from a 50 ml. burette to the cannula. By means of a spring clip on the rubber connecting tube 2 or 3 mls. of saline were run into the vein after each injection.

The blood pressure was obtained from either carotid artery using the regular mercury manometer. Non-distensible plastic tubing was used to connect the cannula to the manometer and also to a reservoir containing 3.8% sodium citrate.

Both femoral and carotid cannulae were siliconed to help prevent the formation of clots. The siliconing technique was first described by Jaques, Feldsted and MacDonald (1946).

The Assay

Several small doses of histamine were first given to determine the sensitivity of the preparation which usually responded by a small deflection to doses of 0.1 - 0.3 µg. Because of this low sensitivity, about 100 times less than that of the guinea pig preparation, the amount of unknown solution given was usually 1/2 or 1 ml. For comparison, a 10^{-6} histamine solution was employed and differences in the volumes injected made up by the saline wash. Injections were made at constant intervals, usually every two minutes.

Atropine sulphate was used to exclude the possible effects of acetylcholine or choline in the samples to be assayed. The dose of 1 mg./ Kg. was given immediately after the chloralose injection.

Neoantergan maleate was also used to prove the presence of histamine in the samples tested. 4 mg. / Kg. was the usual dose given and this was injected just before the test was to be made following the com-

parison of samples with histamine in the usual manner. An example of the assay and neoantergan test is seen in Figure 5.

(c) The Chemical Assay of Histamine

The technique used by Graham, Lowry and Harris (1951) for the chemical estimation of histamine was tested in preliminary experiments but, in my hands, was not satisfactory for routine determinations. Besides its inherent difficulties, the method was not sensitive enough, and the biological methods already described gave more reproducible results. Other methods (McIntyre, Roth and Shaw, 1947; Lubschez, 1949; Roberts and Adam, 1950; Born and Vane, 1952) for the chemical estimation of histamine were not tried.

3. The Estimation of Heparin

The colorimetric method of Jaques, et al. (1947) was employed as a simple test for estimating the amount of plasma heparin in a number of experiments. The procedure is a modification of the original method which used the Lovibond Tintometer, and was adopted for the Beckman Spectrophotometer by Ashwin (1950).

One ml. of the plasma to be assayed is added to 4 ml. of a phosphate buffered solution containing 3 mg. % of Azure A. dye. This solution is twirled and poured into the cuvette which is placed immediately in the Spectrophotometer. The reading obtained is subsequently matched against a standard heparin curve prepared from the same dye solution. Up to 0.1 mg. of heparin per ml. of undiluted plasma can be measured by this technique. Control readings with normal plasma and the buffer solution are also taken so that corrections can be made.

On addition of the heparin, the maximum wave band of the dye at 620 mμ is reduced and a new, weaker one appears at 520 mμ. This

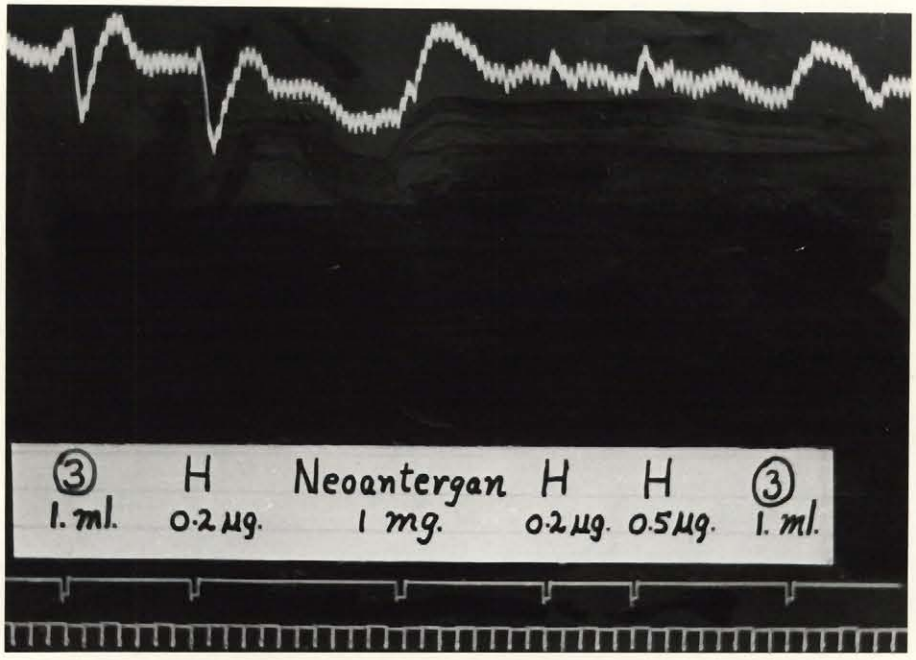


Figure 5.

The assay of histamine by means of the cat arterial pressure preparation. Matching depressions in the blood pressure shows that plasma sample 3 has a histamine equivalent of 0.2 ug./ml. The abolition of these responses by Neoantergon further indicates that the sample contains histamine.

Time scale = 10 sec.

shift is proportional to the heparin concentration. The metachromatic value is therefore determined by measuring the increase in light absorption at 520 mμ. Maximum sensitivity however is obtained at a wave length at 500 mμ. (Ashwin 1950). At this wave length the absorption due to the dye increases linearly with heparin concentration.

4. Blood Pressure Determinations

Blood pressure records were usually made in the ordinary way with the mercury manometer and sodium citrate pressure system. However, because of the possibility that sodium citrate may interfere with the normal mechanism of histamine release its exclusion from the system was obligatory. Rocha e Silva (1952) has shown that citrate is apparently able to stop the process by which histamine is released in some allergic conditions.

In order to prevent the backflow of citrate into the animal, a modification of the apparatus devised by Willbrandt and Cuadra (1947) was used. (Figure 6). By means of a rubber tambour, the blood pressure of the animal operates the escape of air from a closed mercury manometer unit. As the pressure rises in the animal the dome of the tambour, which rises not more than 2 mm., causes a damming in the air system and the mercury rises. A fall in blood pressure permits the air to escape and the mercury to fall. With careful adjustments of the air pressure and tambour the blood pressure can be recorded as faithfully as with the regular manometer. Calibration with a standard citrate-mercury manometer is necessary whenever this device is used.

With this apparatus it is estimated that not more than 0.5 ml. of citrate solution enters the animal's circulation even after the greatest pressure variation. Examples of records made with this device will be found in the experimental work. (see Figure 10).

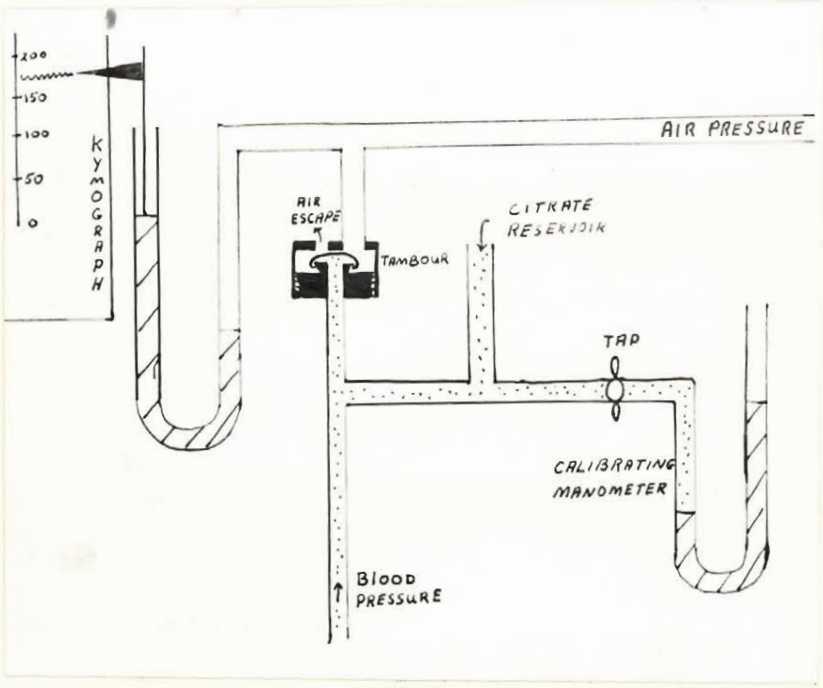


Figure 6.

'Air pressure-recorder' adapted for taking arterial blood pressures. Undulations of the tambour caused by blood pressure variations, regulate the manifest pressure of a stream of air. The air stream operates a mercury manometer, calibrated for arterial pressure and arranged with a stylus for writing on a kymograph drum.

B. THE CHARACTERIZATION OF HISTAMINE LIBERATORS

At the beginning of the experimental period a small supply of the compound 48/80, prepared by Baltzly, Buck, de Beer and Webb, (1949) was received from Dr. de Beer of the Wellcome Research Laboratories, New York. In the accompanying letter from Dr. de Beer mention was made of several of the properties of 48/80 and we believed that it probably was a typical histamine liberator. To confirm the nature of this substance a number of preliminary tests were performed so that a basis for later experiments could be established. I also felt that this was necessary to ensure that the specific effects of histamine liberators, as described by earlier workers, were reproducible in my hands. It has been suggested that the criteria used by MacIntosh and Paton (1949) were not very specific (Alles, et al, 1953).

As a result of these preliminary tests another feature has been found which could be used to characterize histamine releasing substances. This is illustrated in part 4 below.

In the meantime other groups at Mill Hill (Feldberg, Paton, Schachter, and Smith) and University College, London (Mongar and Schild) had also received supplies of 48/80 and found it to have a typical, and very powerful, histamine-releasing action. Most of their experiments have already been mentioned. The present section deals with a number of easily illustrated characteristics of histamine liberators.

(1) The Delayed Effect

The injection of 0.1 mg. of 48/80 into a chloralosed cat causes a sharp and profound fall in the blood pressure beginning about 20 seconds after the injection. (see Figure 7 (b)). The amount of the

drug does not influence this time of delay which remains the same for each animal, although variations are observed in different animals.

(2) The Comparison to Histamine

Small doses of 48/80 cause a histamine-like deflection in the blood pressure but larger doses cause a prolonged depression quite unlike the effects of proportionate doses of histamine, which usually do not last more than one or two minutes.

The dose-response curve for a histamine liberator, unlike that for histamine, is extraordinarily steep in the neighbourhood of threshold: starting with subthreshold doses often an increase of only 10% may cause a sharp fall in the arterial pressure. Double the threshold dose of the histamine liberator is usually able to lower the blood pressure to less than 25% of its original value. Thus, extremely small changes in the dose of the histamine liberator may seem to cause disproportionate variations in the response. For this reason the investigator must give exactly the same dose in just the same manner if responses are to be identical.

In the figure (7a,b,c) a comparison between doses of histamine, 48/80 and diamino-decane diHCl (DA_{10}), a typical histamine releaser, (MacIntosh and Paton, 1949) can easily be made. Approximate equal doses for the three are respectively: 1.25µg., 15µg. and 4 mg. Expressed as a ratio this is 1:12:3200. All injections in Figure 7 were given at intervals of 10 minutes.

(3) The Additive Effect

Whatever other specific actions they may have, histamine liberators apparently cause this common effect by activation of the same mechanism. This is illustrated in Figure 7 where small doses of two chemically dissimilar liberators 48/80 (b) and DA_{10} (c) may add up when given

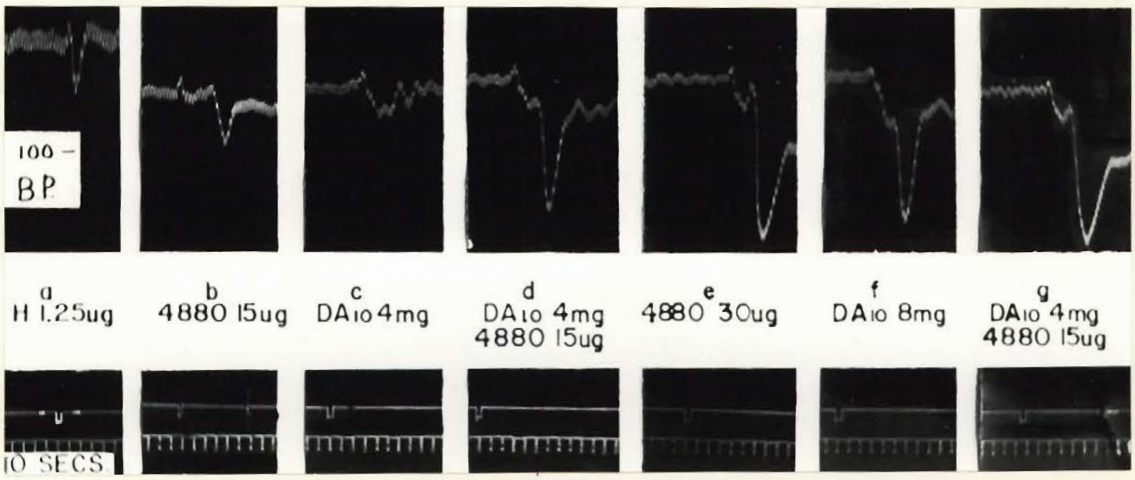


Figure 7.

The action of two histamine releasing drugs alone and together on the cat arterial blood pressure. Cat, 3Kg., Chloralose. For explanation please see the text.

together to produce an effect (d) (g) equal in value to those produced by double values (e) (f) of their own original doses (b) (c). The effect seems even more remarkable when subthreshold doses of each drug add up to produce a threshold response. From this illustration it can be assumed that other histamine liberators could add their effects to those of DA_{10} or 48/80 to produce a heightened response. Also, if it was desired to test a substance for histamine releasing activity this technique would provide a means for quick characterization. As will be seen in the chapter on protamines this technique has already proved a very valuable tool for finding new histamine releasers.

(4) The Delayed Additive Effect

If the liberating compound does not have a concentration sufficient to cause the release of histamine then it has no detectable action at all. (Figure 8 (a)). When the circulating concentration is raised by subsequent injections (b), a normal latent period is followed by the usual blood pressure drop. A third injection (c) shows that the previous two doses, in causing the release of histamine, have themselves been destroyed. The mechanism of this disappearance has not yet been discovered. Because of the possibility that the additive effect may produce erroneous responses, injections of liberators, either the same one or others as was mentioned in part(3) must be given not more often than every 10 minutes.

With the methylated liberator ME_4DA_{10} the approximate rate of destruction was estimated by injecting constant doses every 1 minute or 40 seconds. (see Figure 9). In the record shown, a single dose of 2 mgs. seems to be completely removed from the circulation in exactly one minute.

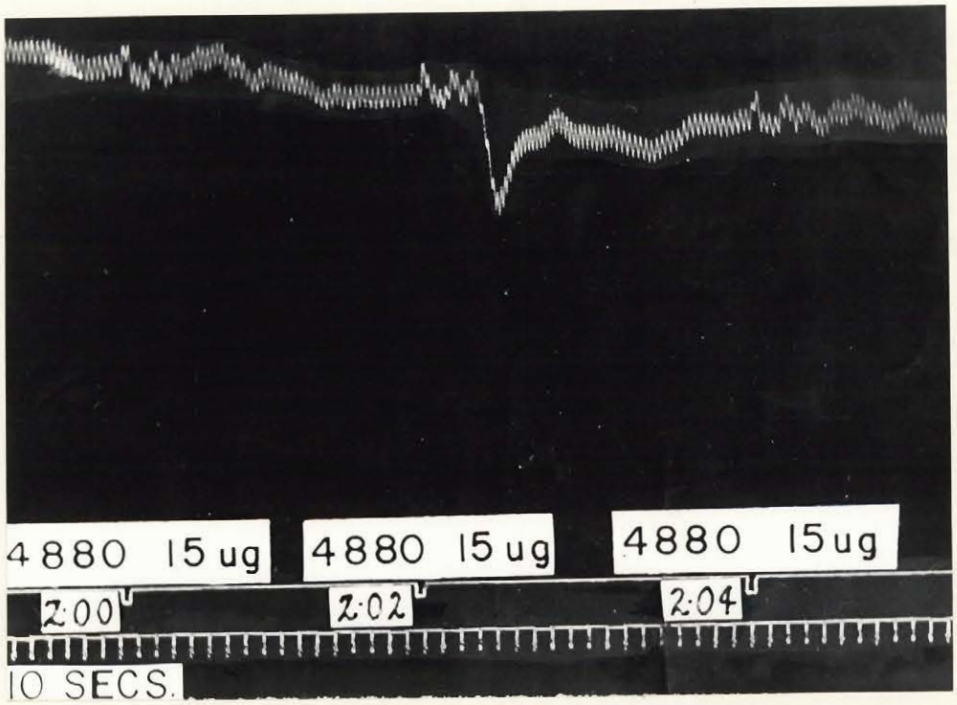


Figure 8.

The effect on the chloralosed cat's arterial blood pressure of administering 48/80 at short intervals. See text for details.

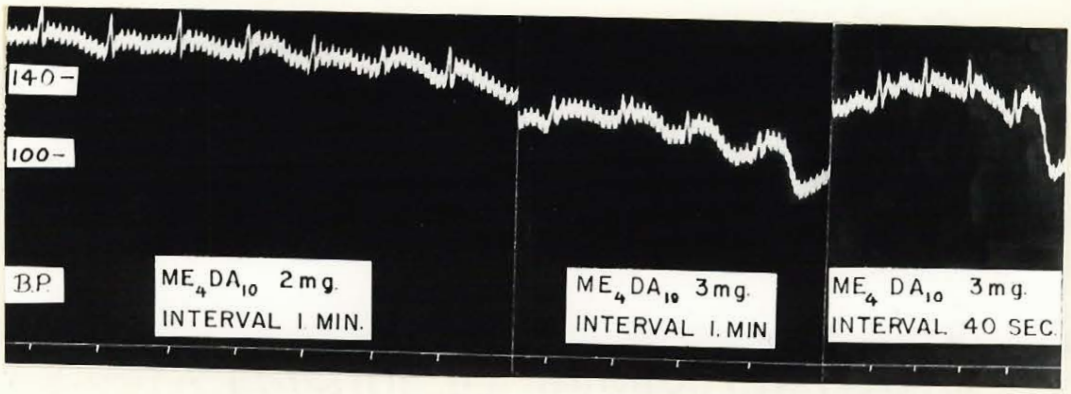


Figure 9

The effect on the blood pressure of a chloralosed cat of administering Me_4DA_{10} at short intervals. The first section shows that 2 mg. of Me_4DA_{10} given every minute has little effect on the animal. When 3 mg. doses are given at this interval a fall occurs after the 4th dose. When this dose was injected at 40 seconds the response occurred more sharply after the 4th dose. The rate of inactivation of Me_4DA_{10} can be determined to be about 2mg./ minute.

(5) Inhibition by Antihistaminics

MacIntosh and Paton (1949) found that anti-histamines prevented the fall in blood pressure caused by small doses of releasers. Dews and co-workers (1953), however, could not demonstrate such a reduction in cats of the pressure fall caused by 48/80. Their dose of 10 mg./kg. of Neoantergan blocked the effect of 0.1 mg./kg. of histamine but not that of 0.05 mg./kg. of 48/80. Using about 1/3 of these doses of Neoantergan and 48/80 we were able to block the fall in blood pressure (see Figure 10). When a second injection of 48/80 was given 10 minutes after the one which followed the Neoantergan, a pressure fall was again observed. If Neoantergan loses its potency thus quickly then Dews' results are easily explained. In his experiment the Neoantergan was given in small doses at a time almost 30 minutes before 48/80 was given.

Another possibility presents itself with the knowledge that Neoantergan may also cause the release of histamine (Arunlakshana 1953). The fall in blood pressure which usually accompanies injections of Neoantergan is a histamine releaser, then it too must be able to add with other histamine liberators and cause an increased response. It is not known why this apparently did not happen in our experiment where Neoantergan was followed by 48/80 two minutes later. On the basis that histamine may also be released by anti-histaminics the works of MacIntosh and Paton (1949) and Dews and his associates (1953) come under criticism: The inhibition of histamine effects with a substance that may also cause the release of histamine should not be permitted to be used as a method for characterising histamine releasing agents.

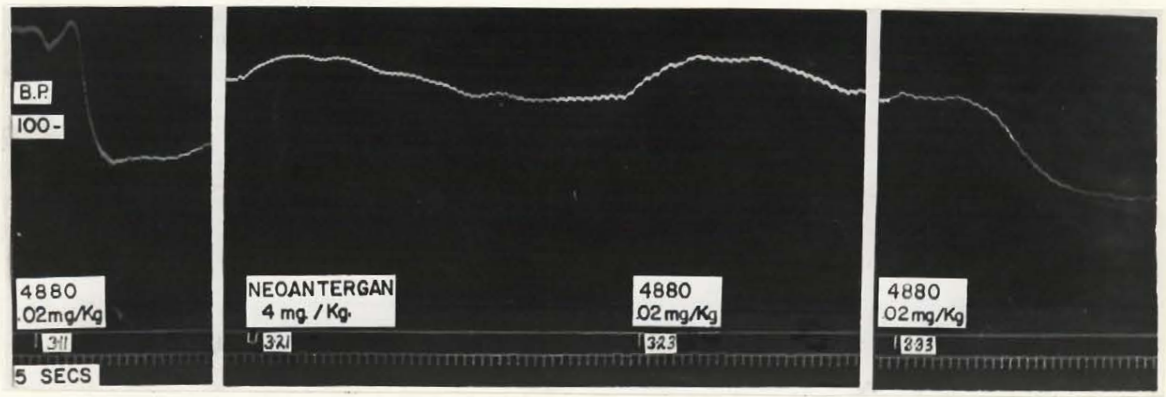


Figure 10.

The effect of neoantergan on the action of 48/80. The inhibition caused by neoantergan has begun to pass off 10 minutes after the administration of neoantergan. Chloralosed cat. The record was made by using the 'air pressure-recorder'.

(6) Skin Reactions

Lewis in 1927 first used the "triple response" to classify various chemicals which released a histamine-like material when they were pricked into human skin.

Pin pricks were made through 10^{-3} solutions of 48/80 and histamine on normal human skin and skin which had received small intradermic injections of neoantergan. The resulting flards and wheals were identical for both substances but they were smaller at the site where neoantergan had been given. Control pricks made through saline produced very minute red areas which soon disappeared.

MacIntosh and Paton (1949) had found similar results for a large group of compounds having other histamine releasing properties. Their extensive investigation has shown that skin tests can be used as a reliable index of histamine releasing activity.

Perhaps MacIntosh and Paton (1949) (and also myself) would not have injected these compounds so boldy under the skin if they had first done skin tests on guinea pigs. When 25 mg. doses of DA_{10} were given subcutaneously on the flank to guinea pigs, an inflammatory reaction appeared at the site of injection within 48 hours. In another day or so the area necrosed with the formation of an abscess. Later a scab formed and retracted from the healthy neighboring area exposing subdermal tissues. In some cases ulceration of the abdominal wall occurred (see Figure 11).

Neither DA_{10} or Me_2DA_{10} appeared to have an immediate general reaction on the animals and up to 200 mg./Kg. given subcutaneously was well tolerated.

Using a very few animals a rough dose - response estimate was made. The following data was recorded when one ml. of various con-

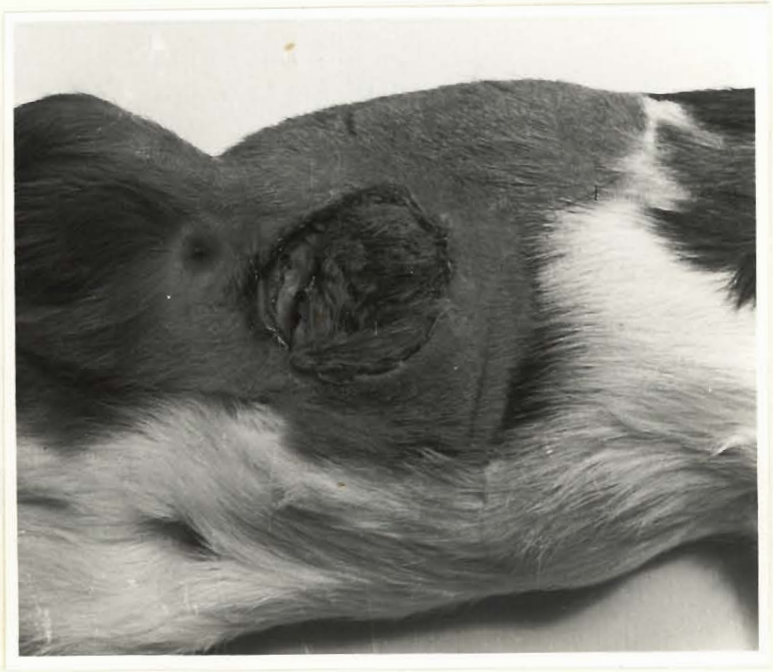


Figure 11.

The effect of 25 mg. DA_{10} given subcutaneously to a guinea pig. These pictures were taken 8 days after the injection. See text for details.

centrations of Me_2DA_{10} were given subcutaneously to groups of two guinea pigs.

Dose of Me_2DA_{10} (mg.)	5		15		25		35	
Wt. of Animals (Gm.)	199	182	164	184	213	202	163	213
Size of Necrosis (sq.cm.)	-	-	1.6	0.7	1.7	4.9	5.4	2.25

When Me_2DA_{10} was given intradermally, smaller doses produced lesions not unlike those produced by subcutaneous injections, but much smaller. With 10 mg. given in 0.1 ml. of solution necrotic areas averaging 0.2 sq. cm. were produced in 18 hours. These were surrounded by inflammatory halos up to 1.5 sq. cm. Doses as small as one mg. of Me_2DA_{10} in 0.1 ml. produced no necrosis but there was a small area of inflammation up to 0.2 sq. cm. (Figure 12.)

Autopsies on animals killed by histamine liberators always showed massive hepatized zones in the lung tissue. No other tissues seemed to be affected. The cause of death in these animals seemed to be due to respiratory conditions. In other animals killed by a blow on the head, the lungs usually showed a few small scattered areas of haemorrhage.

When doses less than those necessary to produce lesions were given subcutaneously every other day for a week, no effects were noticed, but at autopsy a few of these animals showed small haemorrhagic areas in the lungs.

Although DA_{10} and Me_2DA_{10} have very similar actions when injected into guinea pigs, this does not apply to 48/80. Various doses given once or for a number of alternate days produced no lesion at the site of injection. It was found that injections of 5 mg./Kg. or greater were usually fatal within one hour. Such symptoms as tremor, respiratory

Figure 12.

The effect of small doses of Me_2DA_{10} given intradermally to guinea pigs.

Upper animal; 1 mg. Me_2DA_{10} in 0.1 ml. of saline. At 18 hours a small inflammatory area appears.

Lower animal; 10 mg Me_2DA_{10} in 0.1 ml. of saline. At 18 hours an inflammatory halo surrounds a white area of necrosis.

distress, nose scratching, and convulsions usually terminated in the animal's death. Autopsies revealed haemorrhages and hepatization of the lungs. Subfatal doses of 48/80 also produced some haemorrhages in the lungs but there were no other lesions in the body. Usually DA_{10} and 48/80 were injected as the hydrochloride at a pH of 3 to 4, but tests made with solutions made up in 1.3% Na bicarbonate (pH 7-8) showed that the acidity of the solutions injected had no effect on the final lesion.

Because of the similarity of the appearance of the lesion to that described by Boyd (1950) for the Arthus reaction, an attempt was made to prevent the formation of these lesions by the use of Splenin A, (Ungar (1951) has shown that Splenin A was effective in preventing a passive Arthus reaction in the guinea pig's leg joint.) In one test on 6 animals: three of them that had received 100 units of Splenin A on 4 alternate days prior to the injection of 25 mg. of Me_2DA_{10} showed no lesions; the other three responded in a normal way to the compound. However confirmation of this inhibition by Splenin A could no be made when the experiment was repeated three more times.

Drs. Kalz and Telner of the Royal Victoria Hospital have recently been investigating the dermatologic effects of the compound DA_{10}. Thus far, DA_{10} has been shown to be of a little value in treating some allergic conditions, but otherwise its action, as can be seen by means of the "triple response", is very similar to that of histamine. Conditions in which an improvement was found after treatment with DA_{10} included dermatographism, chronic urticaria, interdigital dermatitis and prurigo nodularis (Telner, 1953).

The skin of various animals has been used by a number of investigators (Feldberg and Paton, 1951; Feldberg and Miles 1951) to study

the histamine releasing action of various substances. In cooperation with Drs. MacIntosh and Paton, a technique (Figure 13) first described by Rosenthal and Minard (1939) for the determination of histamine liberation from skin, was performed with only a few modifications. Pieces of skin from normal or sensitized freshly killed guinea pigs were fastened hairy side out, with elastic bands, on the tops of test tubes which had had their bottoms removed. The preparation was then suspended in liquid paraffin at 37°C. One ml. of Locke solution, either normal or containing antigen or histamine releaser could then be placed against the skin which formed the floor of the chamber, and after a measured time be tested for histamine content. In this way the release of histamine could be measured in µg. released/minute/sq , cm. of skin. If desired, subcutaneous tissues could also be removed from the skin section used and placed in a regular test tube for independent estimations. The technique could also be used for the skins of dogs and cats. We did not make very extensive investigations using this technique and reached only a few conclusions.

The method could be used

(a) to measure the release of histamine by antigen or histamine releasers in µg./unit time/ unit area.

(b) to measure the skin histamine of all animals, normal, pathological or sensitized, as it is released by the agent under investigation.

(c) to measure the histamine available for release in different layers of the skin.

(7) <u>The Release of Histamine - Time Relationship</u>.

After large doses of a liberator, heparinized blood samples taken 1/2 to 30 minutes later could be shown to contain varying amounts of histamine. The release seemed to occur explosively because

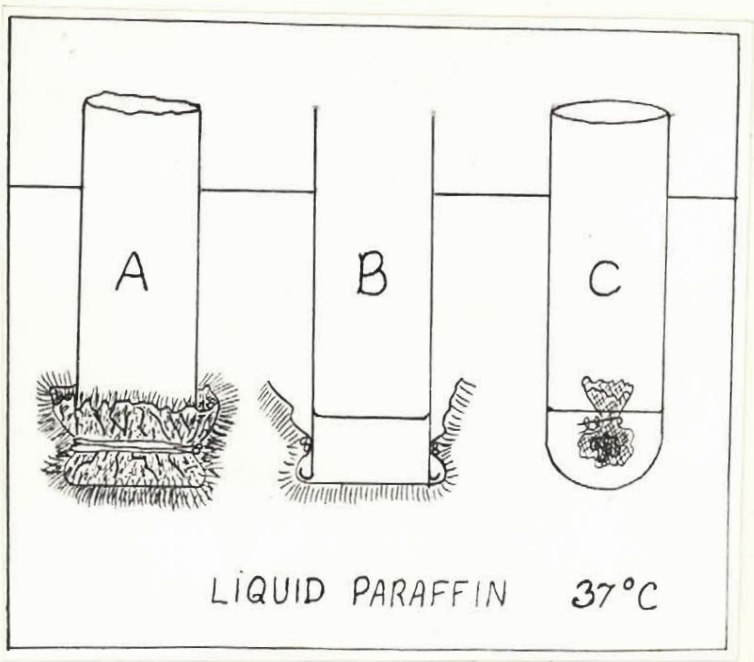


Figure 13.

A method for testing the release of histamine from skin.

A. The skin is attached to the open end of a test tube with an elastic band.

B. Section of A. The skin forms the bottom of a chamber into which the histamine is released. The liquid medium is removed for histamine estimations and replaced with fresh solution every 15 minutes.

C. Subcutaneous tissues can also be tested for the release of histamine by placing them in a gauze bag as shown.

the first samples always showed the highest values of histamine. Feldberg and Paton (1951) showed that the explosive release also probably occurred in perfused isolated skin and muscle preparations. They suggested that the histamine in samples taken after the first few mls. was due to a simple washing-out action of the perfusing fluid.

In the intact animal the rapid disappearance of blood histamine is probably a factor governing the level obtained. When 10 mgs. of histamine were injected into a 13 kg. dog 60% of the histamine disappeared from the blood stream within the first minute. When evenly distributed the 10 mgs. injected by the femoral artery should have given a value of 8µg. per ml. of plasma, but only 3 µg./ml. was found in the most concentrated sample taken from the femoral vein. The results are shown graphically in Figure 14.

(8) The Release of Heparin

Plasma samples taken for the assay of histamine could also be used for the assay of heparin, providing that the anticoagulant used was sodium citrate. The release of heparin was only demonstrated in dogs. To substantiate metachromatic values, clotting times were also recorded. In many instances clotting times alone were used as a rough gauge of heparin released.

The release of heparin also seemed to occur explosively, as could be estimated by the rapid appearance of metachromasia in the plasma samples. The duration of the effect caused by heparin is much longer than that of histamine, however, and this has been attributed to the binding of heparin with serum proteins. (Jorpes, 1946).

Using the fluorescent liberator stilbamidine, Riley and West (1952) have been able to observe the explosive release of heparin from mast

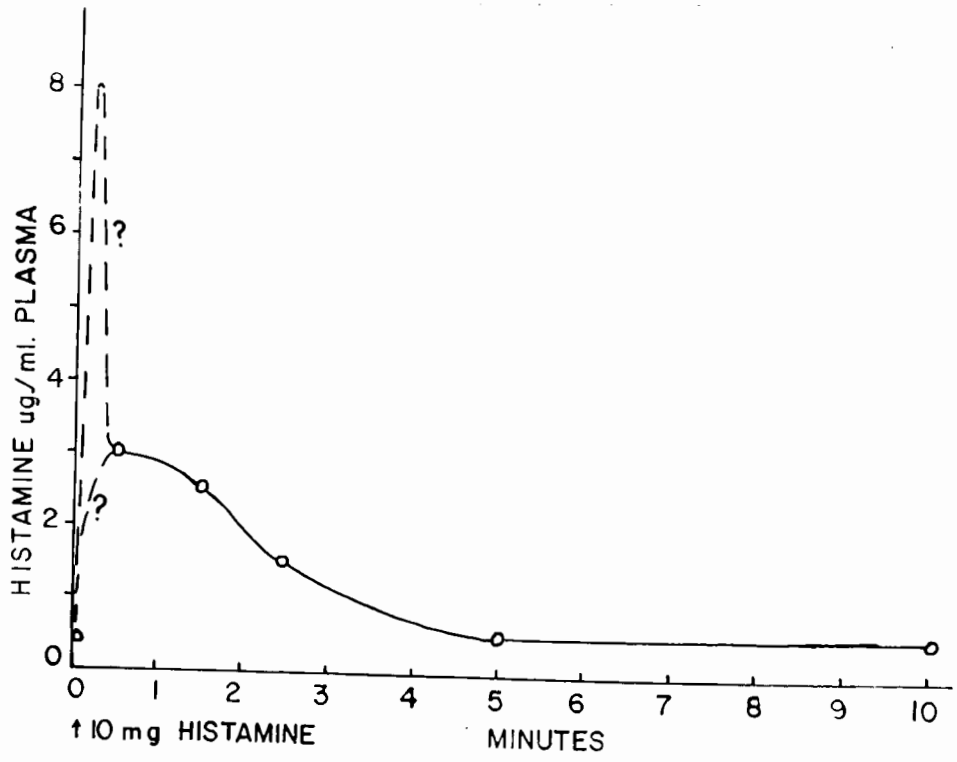


Figure 14.

Plasma levels of histamine following its intravenous injection. Sufficient histamine was injected into a 13 Kg. dog to raise the plasma histamine level to 8 µg./ ml. when evenly distributed in the blood stream. The interrogations indicate the possible levels obtained in the circulation before the 1/2 minute mark. The rapid loss of histamine from the blood stream probably would not permit the 8 µg. level to be achieved.

cells. However, Devitt (1953) has shown that a great number of non-specific methods are available for the release of mast cell granules and has also shown that the potent histamine liberator 48/80 neither reduced the number of mast cells in various tissues of the rat nor had any effect on the state of the granules.

C. THE SITE OF HISTAMINE RELEASE IN THE DOG

By injecting known doses of histamine and comparing the blood levels thus produced with those occurring in anaphylactic experiments, Dragstedt and Mead (1936) found that toxic amounts of histamine were required and that the vascular reactions produced by the histamine injections were almost identical with the anaphylactic reactions to which the blood concentrations of histamine corresponded. Ojers, Holmes and Dragstedt (1941) subsequently showed that the liver was the source of histamine released during the anaphylactic reaction and this fact is now generally accepted. However, Water, Markowitz, and Jaques (1938) have demonstrated that not all anaphylactic manifestations are associated with the liver.

Experiments with peptone by Holmes, Ojers, and Dragstedt (1941) similarly suggested that the liver released shock-producing amounts of histamine.

MacIntosh and Paton (1949) did not estimate liver histamine values in experiments with histamine liberators but were of the opinion that the compounds examined by them caused their effects in the dog by freeing hepatic stores of histamine. It was to test the validity of this assumption that the following experiments were undertaken.

(a) Evaluation of Liver Histamine

Hepatectomies were performed on male dogs of any size, anesthetized with nembutal (1 ml./5Kg.) combined with sodium phenobarbital (0.1 Gm./Kg.). Blood pressure records were not made and blood samples were not taken.

It was found that 3 portions of the liver could be taken without trouble and without excess bleeding. These were: 1. the left lateral lobe, 2. the central lobe and 3. the remaining portion largely composed

of the right lateral lobe. The first and second portions were tied off with strong, wetted cotton cords and then removed with sharp scissors. Each part and its contained blood was weighed and treated for histamine estimation by the method of Barsoum and Gaddum (1935). The last portion was removed without the use of ligatures. The gall bladder was easily separated from this mass and excluded from further treatment.

Portion 1 was usually taken 5 minutes before the liberator was given and used as the control sample. Portion 2 was removed 20 minutes after the injection and this was followed immediately by the 3rd part. Estimations on the 2nd and 3rd parts indicated the amount of histamine lost either by procedure alone or by procedure plus DA_{10} injection.

For most experiments assay was made by both guinea pig and cat blood pressure methods. Atropine and neoantergan were used in all assays. No assays were hampered by the presence of interfering substances.

In the accompanying table (TABLE 1)all data connected with this investigation are recorded. For all experiments DA_{10} was given by the femoral vein in a dose of 1 or 2 mg./Kg. This amount was reduced in later experiments as the larger doses sometimes shortened the animal's life unless artificial respiration was employed. With large doses the actual amount given did not seem to influence the percentage of histamine released.

Liver histamine in the two latter samples was evaluated statistically for both control and experimental runs. The loss of histamine by DA_{10} averaged 19.9% and was significant at the 0.1 % level ($p=<.001$). In the control samples the loss of 3.75% was insignificant ($p\doteq 0.30$).

(b) Blood Levels of Histamine after DA_{10}

Having found that a substantial percentage of liver histamine was lost during shock by DA_{10} we now considered whether the blood histamine content gained during shock would duplicate this value. Estimations of blood histamine were made on plasma samples taken at various times after the injection of the liberator.

In two preliminary experiments using 0.5 mg. of 48/80 /Kg. only 1.8% and 14% of liver histamine losses could be demonstrated in the blood stream two minutes after the injection. This seemed to indicate a very rapid loss from the blood stream of histamine released by the liver. That this was probably true to some extent is supported by our knowledge that at the 2 minute mark after a large dose of histamine (see Figure 14) only about 20% of the histamine injected remained in the blood stream.

For one experiment with DA_{10}, the liver was shown to have lost a total of 289 µg. of histamine, but only 25µg. could be demonstrated in the blood at the 2 minute mark. By extending the curve formed by values obtained at the 2, 4 and 6 minute marks backward to 0 minutes, a value of 62 µg. could be accounted for. However, the steepness of the curve in the region of extrapolation forbids accurate estimations to be made.

By taking samples every 15 seconds after 1.0 mg./Kg. of DA_{10}, a total peak value at 45 seconds of 67 µg. histamine was estimated. No loss could be demonstrated for the liver, where every sample assayed at 23 µg/Gm., but this is not very surprising as 67 µg. represents less than 1% of total liver histamine and this would be extremely difficult to measure.

In another experiment the portal route was used to inject the drug. This time it was found that the peak level was reached at 20 seconds.

TABLE 1

THE LIBERATION OF HISTAMINE FROM THE LIVER WITH DA_{10}

CONTROL									EXPERIMENTAL								
No.	Liver Sample			Liver as	Histamine µg./Gm.			Liver	No.	Dose	Liver Sample			Liver	Histamine µg./Gm.			Liver
Weight	No.	Weight Gms.	% Total Liver	% of animal	Cat	G.Pig	Ave.	Histamine % of No.1	Weight	DA_{10} mg.	No.	Weight Gms.	%Total Liver	as % Dog	Cat	G.Pig	Ave	Histamine % of No.1
143	1	60.6	22.4	3.0	39	31	35	--	146	27	1	48.4	27.4	2.6	36	44	40	--
9 Kg.	2	16	5.9		23	25	24	67	6.7Kg.		2	21.1	12.0		28	34	31	78
	3	19.2*	71.7		30	27	28	82			3	106.1	60.6		32	32	21	78
144	1	67.5	26.2	4.1	11	11	11	--	147	17.5	1	72.2	29.8	2.8	43	35	39	--
6.3Kg.	2	23.2	9		12	12	12	109	8.6Kg.		2	22.4	9.15		36	30	33	84
	3	166.9	64.7		11	11	11	100			3	147.1	61		27	23	25	64
148	1	88.8	29.2	3.4	26	--	26	--	149	19	1	78.4	26	3.3	46	--	46	--
9 Kg.	2	36.7	12.1		24	--	24	92	9.5Kg.		2	36.1	12		45	--	45	96
	3	178.5	58.7		22	--	22	84			3	186.3	62		40	--	40	89
150	1	56	23	3.4	16	20	18	--	151	5.5	1	19.8	11.1	3.4	42	46	44	--
7.2Kg.	2	32.7	13.5		16	16	16	99	5 Kg.		2	44.1	24.6		34	34	34	78
	3	154.5	63.5		17	23	20	109			3	107	65.3		34	34	34	78
153	1	82.2	27.2	3	11	--	11	--	152	6	1	49.5	32.4	2.3	14	--	14	--
10 Kg.	2	42.9	14.2		12	--	12	109	6.5 Kg		2	24.5	16.1		11	--	11	83
	3	176.6	58.6		11	--	11	100			3	78.4	51.5		10	--	10	75
207	1	47	31.5	2.3	--	24	24	--										
6.6Kg.	2	21	14.1		--	23	23	97										
	3	81	54.4		--	24	24	101										
Averages				3.2			21.1 18.8 19.7	-- 95.5) 96) 96.25						2.9			37 31 28	 83.8) 76.4) 80.10

* A forth portion of the liver was not used for assay.

Loss of histamine due to procedure = (100-96.25)= 3.75%
Value of t for this difference = 1.03
p = 0.30, S.D. = 12.75

Loss of histamine due to DA_{10} = 19.9%
Value of t for this difference = 6.33
p = < 0.001, S.D. = 9.73

The difference in times taken for the peak to be reached after femoral and portal injections can easily be accounted for if the liver is assumed to supply the histamine released. However our plasma histamine values still only accounted for 1.4% of liver losses.

In another dog the intraportal injection of 3 mg/Kg. of DA_{10} produced plasma levels of histamine much greater than could be accounted for by direct loss from the liver. According to Dr. MacIntosh the value of 12 µg./ml. plasma established a new record for histamine released after injection of a liberator. For the 10.2 Kg. dog this level, which reached its peak at 20 seconds, would give a total of 4896 µg. of plasma histamine. (Dog plasma value = 4% Body weight : Best and Taylor).

The loss of histamine from the remaining 2 sections of liver amounted to 2778 µg. - an average loss of 43% of their normal content of 42 µg./gram. The discrepancy in values can be accounted for by 2 possibilities.

1. The sample containing 12 µg./ml. may have been taken at the crest of histamine just released and would naturally be above the average level in the circulation.
2. DA_{10} may have released histamine from sources other than the liver; e.g. the lung.

The second of these suggestions would necessarily involve the lung or heart as they are the only organs situated between the point of injection and point of sampling. It is also necessary fo assume an explosive release of histamine from its source so that the peak would accur sharply at the 20 seconds mark. The other obtained values are recorded below:

Time (seconds)	0	5	15	20	30	50	115
µg./ml. (guinea pig)	.01	.03	4	12	9	5.2	5.2
µg./ml. (cat)	unmeasurable		4	12	10	10	10

The other reason seems to be more satisfying, but it is also difficult to estimate the time when equilibrium in the blood stream is reached. Even if this was obtainable, an unknown amount of histamine would have been already lost from the blood stream.

Another difficulty is the question of how much released histamine is destroyed by the liver itself. Anrep, Barsoum, and Talaat (1953) found that the liver was able to remove good amounts of histamine from the blood stream. The loss of liver histamine via the hepatic lymphatics, as shown in some earlier experiments, is also possible. (Dragstedt and Gebauer-Fuelnegg, 1932).

For these reasons it was decided to forego further attempts at liver and blood histamine correlation.

(c) Histamine Release after Portal Shunt

The first two animals did not have a proper portal shunt but the liver was isolated by means of firm clamps on all vessels entering the liver. In the table the results of two injections with and without the liver in the circulation are recorded.

Liver not in Circulation

Time (Seconds)	0	10	20	30	70	600	1200
Histamine (µg./ml.)	.015	.042	.032	.029	.042	.036	
Liver Sample No. 1	↑ 56.5 µg./Gm.						
Liver Sample No. 2							↑ 53.4µg./Gm.

Arrow indicates time of liver sampling.

Liver in Circulation

Time (Seconds)	1200	1210	1220	1230	1245	1265	1325	1800
Histamine (μg./ml.)		.018	.026	.030	.030	.045	.022	.035

Liver Sample No. 3 35.2 μg./Gm.

Although extremely small increases were found for both runs the participation of the liver during the 2nd part is established by the loss in liver histamine during the procedure. Even with only 1/3 of the liver available for release during this run the total amount of histamine released (666 μg.) compared to that recovered (30.6 μg.) seems somewhat improbable.

In another dog the great increase in blood histamine caused by DA_{10} could not be accounted for by release from the liver which was again clamped out of circulation. Liver values remained constant at 64 μg./Gm. but the plasma histamine rose to 0.5 μg./ml. at the 3 minute mark, as shown.

Time (seconds)	0	30	45	60	180	600
Histamine (μg./ml.)	.01	.033	.087	.325	.520	.100

The reason for this delayed output of histamine is not known. However the period between the 1 and 3 minute marks was not sampled so no judgement regarding the manner of release can be made.

In this experiment, when the clamps were released and the circulation appeared to have returned to normal, a second injection of DA_{10} failed to bring about a rise in the plasma values of histamine. The only indications of a reaction were engorgement of the liver and an apparent fall in the blood pressure. Unfortunately, no third liver sample was taken, but all other observations indicate that the histamine in first run was released from sources other than the liver.

Reversing the procedure, the initial injection of DA_{10} (2 mg./Kg.) intraportally, released a total of 1680 µg. of histamine. Then by means of a siliconed glass shunt (Figure 15) the portal blood was transferred to the inferior vena cava. A hemostat forceps was clamped on the liver hilus to ensure no circulation through the liver.

During both injections the blood pressure was taken. With each injection a sharp fall occurred but there was no recovery after the second dose of the liberator. In the second run 140 µg. of histamine was found in the blood stream at the height of the response. The plasma values were as follows:

Liver in Circulation

Time (seconds)	0	15	25	35	60	90	600	5400
Histamine (guinea pig µg./ml.)	.025	.99	2.14	2.4	2.4	0.5	0.2	0.075
(cat)	-	1.1	3.2	3.5	1.4	0.5	trace	-

Liver out of circulation

Time (seconds)		15	30	50	70	120
Histamine µg./ml.	(guinea pig)	.10	.20	.14	.1	.1
	(cat)	----------trace----------				

Employing the same glass shunt, 2 more trials were made with the liver excluded from the circulation. Blood samples were taken before the injection and at intervals after the injection of 3 mg./Kg. of DA_{10}. The results for one dog are tabulated below:

Time (Minutes)	0	1.0	1.5	2.5	3.5	10
Histamine µg./ml.	0	0.030	0.340	0.160	0.100	0.050

In the samples at 0 and 1 minutes small contractions were shown to be due to "slow-contracting substance". Samples from the other dog

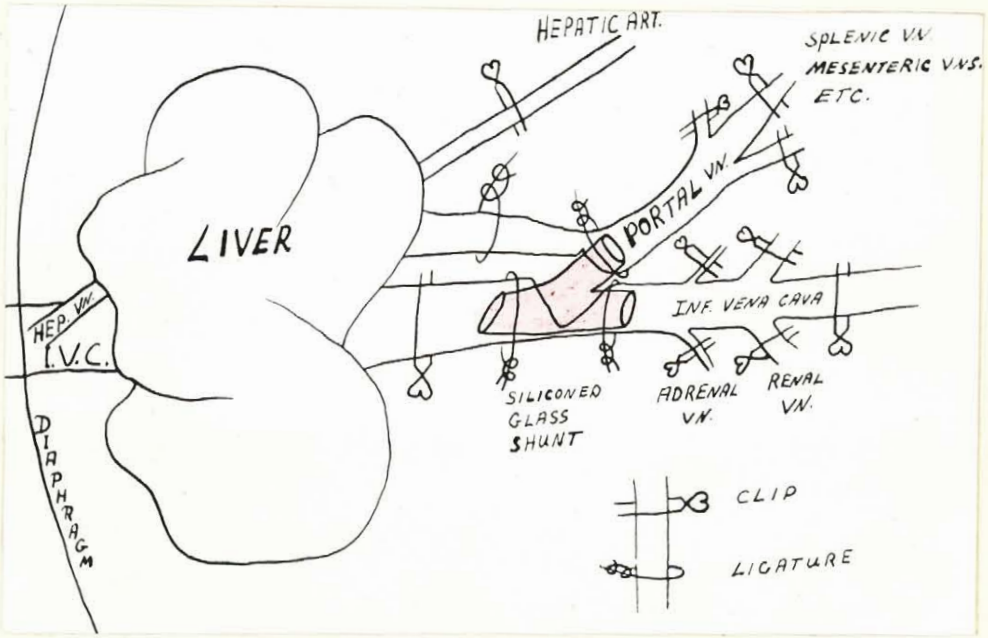


Figure 15

Diagram to show the portal shunt used to exclude the liver from the circulation. The shunt is made of glass, siliconed to prevent clots. After the vessels are dissected clear, the shunt is inserted in two steps. (1) The portal vessels are ligated or clipped and the portal vein cannulated. (2) The caval vessels are clipped and the other two arms of the shunt tied in place. The clips are then removed and the blood allowed to pass through the shunt. The operation is a simple one. The blood flow is usually not interrupted more than 10 minutes.

did not contain any histamine and only traces of "slow-contracting substance" were found.

The fact that liver anoxia may have been produced in the above experiments has not been neglected. This is discussed in a following chapter.

Conclusions:

(a) Significant amounts of histamine are lost from the livers of dogs shocked with DA_{10}

(b) Histamine may also be released from other tissues of the intact dog during the shock caused by histamine liberators.

D. REFRACTORINESS TO HISTAMINE LIBERATORS

In the preceding chapter it was shown that the release of histamine, even with large amounts of liberators, may vary in whole animals from extremely low to very high values. When a dog is found who responds very poorly to the actions of histamine releasers one is tempted to call the animal "refractory". MacIntosh and Paton have also mentioned that in a number of dogs the effect of their histamine releasing compounds was either absent or much reduced.

The only explanations so far possible seem to be that the animal's supply of histamine readily available for release is either naturally low, or else has been previously depleted by the administration of histamine liberators. An example of the former may be taken from the work of Mongar and Schild (1952) who found that very little histamine could be released from the intestine of guinea pigs, in comparison to that released from other tissues. The small intestine, especially, could almost be classed as being naturally "refractory". However, in whole refractory animals every tissue must possess this queer property or else, when released, the histamine must be destroyed almost immediately.

The well-known refractoriness to peptone of dogs just recovered from peptone shock was found by MacIntosh and Paton (1949) to extend to the effects of histamine liberators, for both histamine releasing and clotting time effects. However, many investigators have noticed that some dogs are from the beginning insensitive to peptone or liberators and appear to exist that way, in perfect health, quite naturally.

Since no explanation had been offered for this state of refractivity, we therefore turned to study the phenomenon as one means of

approach to the study of histamine liberators.

In the section on histamine release a number of methods were shown to be available to prevent various histamine releasing reactions (e.g. anaphylactic) from reaching fatal proportions. Perhaps the use of antihistaminics was the most successful of these, but it was still only able to partially prevent the symptoms caused by the histamine released and did not depress the release of histamine itself. In contrast, if a method is able to prevent the onset of the anaphylactic reaction in an animal, the animal can be said to be "refractory". (Actually the term "refractory" has only been applied, by us, to animals that are naturally so. "Induced refractoriness" was to be applied to animals in which the release of histamine could be successfully prevented).

It is well known that dogs who have lived through a major anaphylactic reaction are usually refractory to further injections of the antigen. This first intense reaction and the following refractioness have posed major difficulties in the study of anaphylactic and allergic phenomena. Since it is almost impossible to regulate the degree of shock produced by the specific antigen, the animals can rarely be used more than once for any experiment. For a limited numbers of experiments involving the study of anaphylasis, the great variability of the reaction usually necessitates large numbers of animals, like the dog, which cannot conveniently be supplied in large number and of a pure strain.

In the introduction it was emphasized that a great many similarities exist between the reactions seen in anaphylactic reactions and those caused by "histamine liberators", either in whole animals or isolated tissues. It was also pointed out that whereas the anaphylactic reactions

could be regulated only with difficulty, those caused by the histamine liberators were easily controlled and reproducible.

It was already mentioned that the number of useful drugs that, as a side action, have the property of histamine release is already considerable and is steadily increasing. The practical significance of the problem was thought to justify the investigation of possible prophylactic measures even when the logical basis for trying them out was rather tenuous. Two facts that have already been referred to (page 116) made this somewhat random exploration appear more hopeful:

1. The fact that dogs surviving severe shock induced by histamine liberators may be almost or quite refractory to further treatment with the histamine liberator, and,
2. a small proportion of dogs, initially distinguishable in no way from the general run of dogs, are found to be insensitive from the beginning. (see Figure 16).

1. Attempts to Characterize Refractory Animals.

In our earlier experiments and in the studies of MacIntosh (personal communication) about 5% of all dogs appeared to show the peculiarity of refractoriness. As the kennels were supplied with dogs, therefore, we planned to characterize all the animals and set aside refractory ones.

Initially, we wished to establish a method for characterization that would not injure the animal or make it useless for another investigation. We believed that this could be done quickly and easily by making a single injection of liberator and noting whether or not a fall occurred in the blood pressure.

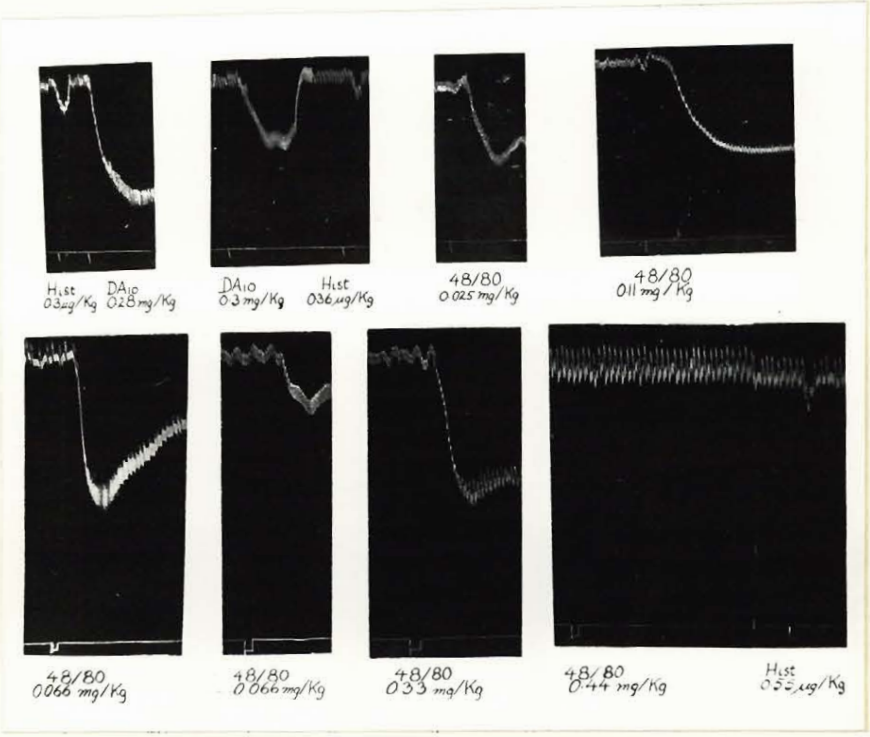


Figure 16

Responses of 8 dogs to 48/80 or DA_{10}. The lower right record shows that the usual response to a moderate dose of a histamine liberator was not obtained. The records show that up to a 10-fold increase in the amount of the liberator is completely ineffective in the refractory dog. Approximate ratio of refractory to non-refractory dogs = 1:30.

A few trials taught us that some animals would not cooperate and would have to be anesthetized before testing. Even with this help pressure readings by palpation or ausculation was difficult because with small doses of liberator the duration of the fall was too brief and with large doses the pressure may be so low that no measurement could be made at all.

By means of a 20 gauge needle inserted into the usually anesthetized animal's femoral artery the arterial pressure could be measured in one of two ways.

1. A tambour citrate pressure system with kymograph recording, or
2. An electromanometer in which the pressure was recorded on a slow moving viso-cardiette graph after modification by capacitor-type transducer.

Either system was found to register pressure variations quite faithfully but clotting usually occurred even in siliconed needles within 5 minutes after their insertion. However no refractory animals were found in a total of 8 attempts.

Supposing that the release of heparin occurred simultaneously with the release of histamine, clotting times were used to determine if liberators were evoking this type of response. Using one 20 gauge needle throughout the procedure 5mls. of blood were first taken from the brachial vein and placed in tubes for clotting times. A 1 ml. syringe was then attached to the yet inserted needle for injection of the liberator and one minute later a third syringe was used to take another 5 mls. of blood. A reasonable increase in the clotting time served to indicate that heparin, and probably histamine, also had been released. The method was certainly a successful one but still no refractory animals could be found. One objection to this technique

is that while concomitant release of heparin and histamine has never been disputed, it is not yet a proven fact.

The behavior of unanesthetized animals after injection of a liberator suggested that this alone could be used as a means for distinguishing those not responding to reasonable doses of the liberator. Any combination of the usual features such as itchiness and nose rubbing, sneezing, weakness, defecation and/or vomiting should readily serve to separate uncharacteristic dogs. The plan seemed a good one but still no refractory animal could be found.

Apparently we had miscalculated the number of refractory animals normally occurring in our kennels or else had been unlucky in our choice of subjects.

2. Attempts to Cause Refractoriness to Histamine Liberators

In the paper by MacIntosh and Paton (1949) it was shown that a number of substances could be employed to depress the response caused by histamine liberators. However the inhibitions caused by calcium salts, heparin, or alkalis were not studied further. The following investigations were carried out with the hope that inhibition of the action of histamine releasers could be obtained. A successful method, if suitable, would be a valuable prophylactic measure against common medicinals whose harmful side actions include the release of histamine.

(a) Miscellaneous Experiments

The technique of testing for refractoriness was a simple one. Thresholds of dogs to 48/80 and histamine were found before and after the treatment and increases in the ratio of 48/80 to histamine indicated the degree of refractivity obtained.

Initially a number of simple procedures were tested once or twice each but without success. These were:

(1) The Administration of distilled water to produce haemolysis.

MacIntosh and Paton (1952) found one dog in which the accidental injection of hypotonic solution had caused extensive intravenous haemolysis. The dog was found to be refractory but the cause of this had not been followed up by them.

(2) The Administration of Indian Ink (1 ml./Kg.).

The reticulo-endothelial System is believed by some immunologists to be of significance in antibody formation and in the genesis of anaphylaxis. It appeared to be of interest to try the effects of "blocking" this system.

(3) The Administration of thrombin (2 mg./Kg.).

The release of heparin and possibly of some protease that is associated with the action of histamine liberators, and the remote possibility that the naturallyinsensitive dogs were those suffering from some thrombotic condition, suggested the trial of thrombin. Thrombin is currently believed to be a proteolytic enzyme with special affinity for specific linkages in prothrombin.

(4) The Manual Traumatization of the Abdominal Viscera.

There was no very good reason for testing this procedure except that it is known that the application of various types of trauma in several species has been followed by increased resistance to stress. (See Selye, 1946).

(5) Typhoid Paratyphoid (A and B) Vaccine.

Typhoid vaccine has been given intravenously for the treatment of asthma and other allergic conditions - often with marked improvements following the chills and fever. (Feinberg, 1949).

It was thought that the reasonable similarity in these conditions to that of histamine release warranted the trial of vaccine.

(6) Acetyl Salicylic Acid (A.S.A.)

No apology is needed for the application of A.S.A. to our studies since it is known to have some beneficial effect on a variety of clinical conditions and also prevents anaphylaxis in guinea pigs. The inhibitory effect of salicylates on the release of histamine and slow-reacting substance in sensitized guinea pig lungs has recently been shown by Trethewie (1951).

(b) Cross Immunity Experiments.

(1) Immunity to 48/80 after large doses of Histamine

By the continuous injection of histamine over a period of several hours Emmelin (1951) was able to raise the threshold of cats to histamine a great number of times. In his experiments up to 1 mg./Kg./ minute of histamine was given.

Using Emmelin's technique, cross immunity between histamine and 48/80 was attempted. Four cats and one dog were used and several milligrams of histamine were infused during periods varying from 1.5 to 3 hours. The effect of this treatment was determined by finding thresholds for both substances before and after the infusion. The histamine threshold was found to have increased an average of 45 times (range; 4 to 160) while the threshold to 48/80 increased an average of 10 times (range 0 to 20).

These values were comparable to other experiments where only a little histamine was injected intermittently and changes of this order were not seen.

The mechanisms thought responsible for these changes were:

(a) for histamine - a reduced ability to evoke a response in the

blood vessels because of their fully dilatated condition; increasingly rapid destruction of histamine and/or neutralization of it by: 1. the kidney (see Emmelin, 1951), 2. histaminase, or 3. other body systems; and

(b) for 48/80 - a reduced amount of tissue histamine readily available for release, or, as above for histamine.

(2) Cross-immunity between "liberator shock" and anaphylactic shock.

Refractoriness of sensitized animals to the specific antigen was first shown by Besredka (1908) and Besredka and Bronfenbrenner (1911) to occur in animals which had just received one dose of the antigen. It was suggested by Topley and Wilson (1929) that such animals were rendered refractory by the saturation of antibodies. On the other hand, "cross-immunity" between peptone, trypsin and histamine liberators seems to be due to the exhaustion of preformed histamine stores in the body tissues (Feldberg and Talesnik, 1953) or else, as Bronfenbrenner (1944) has suggested, to a rise in the serum antitrypsin titre. It has also been shown (Bronfenbrenner, 1915) that the injection of anaphylactoid agents caused animals to become refractory to anaphylaxis and remain so until the raised antitryptic titre returned to normal. (Burdon, 1942).

It was partly to test the application of this last statement to histamine liberators in dogs that the following experiments were undertaken.

Dogs were sensitized to egg albumen using the technique of Scroggie et al (1949). After 30 days, dogs that did not die following several large doses of liberator or egg white were reinjected an hour or so later with the other agent. In all experiments the blood pressure was taken by means of the air pressure-recorder (see Figure 6) and clotting times were observed.

Results in these tests were only taken from those in which the first injections caused good histamine-releasing reponses (indicated by the blood pressure record). In every case, whether the animal was first desensitized to 48/80 or egg albumin, (three experiments of each), an injection of the other substance usually caused a further fall in the pressure. With the second injection, the blood clotting time which had already been raised due to heparin released by the first agent, again rose to values above normal.

One set of plasma histamine levels, estimated before and after the dog no longer responded to injections of egg albumen, showed that this treatment increased the values from a normal of 0.03 µg./ml. up to 0.05 µg./ml. 48/80 then caused a further increase to 1.1 µg./ml.

Thus is no instance could cross immunity between histamine liberators and anaphylaxis be shown.

(e) The Reproducible Threshold-Response Technique

In order to measure smaller differences caused by influences modifying the release of histamine, our technique was modified to a manner suitable for statistical analysis. The threshold dose of the liberator was established as before, but now it was given intermittently every 10 minutes. Histamine controls were given every 10 minutes midway between the liberator injections. Threshold doses were taken to be those which caused a fall in the blood pressure of about 20%.

When a constant threshold dose of the histamine liberator could be given 4 or 5 times with very little deviation, the threshold was considered as having been established. The animal was then subjected to the proposed modifying influence and injections of the liberator were continued. Differences in the response were usually very easily

seen and if desired could be treated statistically. Also, any changes could be compared to those observed for histamine. By this technique it would be possible to determine if the effect of the new influence was immediate or delayed and whether it acted strongly and then diminished, or began slowly, to reach a maximum effect some time later.

Control animals (cats) were observed for periods up to four hours. Figure 17 shows the average magnitude of responses obtained in 8 animals treated with DA_{10} and histamine. Table 2 gives a complete account of the data employed together with the blood pressure observed at each interval.

A number of causes are suggested to account for the fluctuations seen. At very high or very low blood pressures the percentage falls are often greater or smaller respectively than those obtained at medial levels. The fall seems to be a function of the blood pressure and possibly reflects the tonus of the capillaries and their ability to relax to histamine. Unfortunately this cannot account for some very large falls occurring when the blood pressure is very low. The major depression in the DA_{10} line which represents the average response to repeated doses of the histamine liberator can be almost wholly accounted for by the values obtained in one experiment. Expt. No. 258 (see Table 2) shows an abrupt change in the depressor response at about the second hour and this again rises at about the 3.5 hour mark. The graph may have been a much straighter line if the responses in this one experiment were more consistent.

The occasional occurrence of a tempory reduction of the response after the injection of any drug under trial, in the absence of any modifying agent, therefore may be of only casual significance. In one

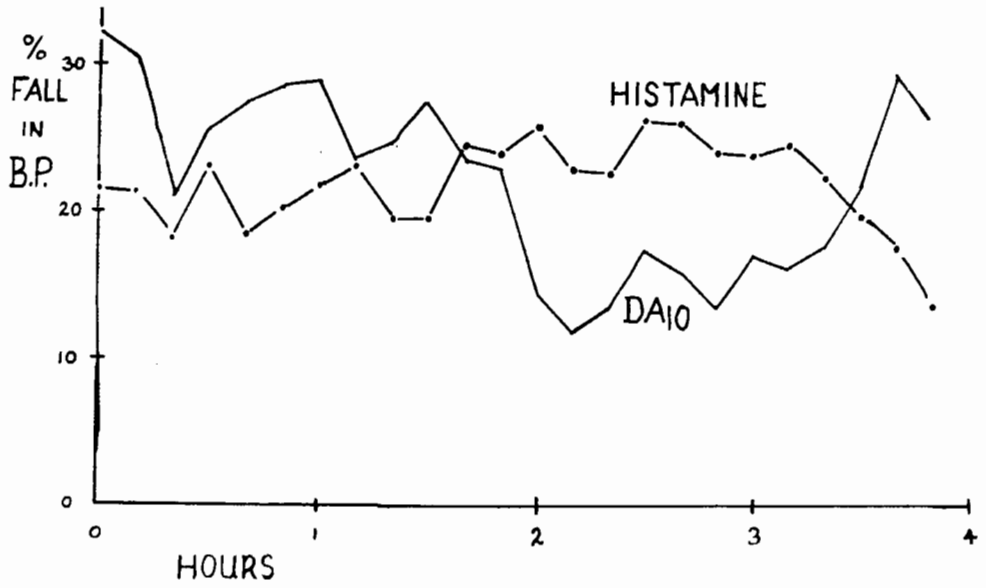


Figure 17

Control responses in cats for threshold doses of DA_{10} and histamine. Injections were given every 10 minutes to 8 cats. Responses were recorded for periods up to 4 hours (see Table 2).

or two experiments, indeed, (e.g. see hydroferulic acid) the first experiment with a new substance appeared to give promise of the sought-for action - a promise that was belied by facts later obtained.

The great sensitivity of many animals to liberators at the beginning of the run may account for greater responses at the left side of the figure. This is believed to be due to the presence of more easily releasable histamine, and, as this supply disappears during the experiment, the animal responds less dramatically.

With this technique then, the following influences were evaluated for their inhibitory effects.

(1) Homologues of Adipic acid

With such simple histamine liberators as those of the straight chain diamine series, we thought that interference by molecules of a similar structure but opposite in charge might be possible. To try this idea, the straight chain aliphatic acids like adipic and its homologues were neutralized with sodium hydroxide and injected into cats.

Figure 18 shows the prompt reduction that occurred in responses to both histamine and the histamine liberator. However, the responses returned to normal values almost as quickly. Because of the antagonism of adipic acid towards histamine, one is unable to judge whether there was any inhibition of the release of histamine by the liberator.

In six more experiments with adipic acid on cats, the pattern of abolition for both histamine and the histamine liberator response was always the same and only once did the effect last for more than half an hour.

Of the other acids tested in this series (sebacic, dodecanoic, azelaic) only sebacic appeared to have very much activity, but its action was not unlike that of adipic and could only be obtained when

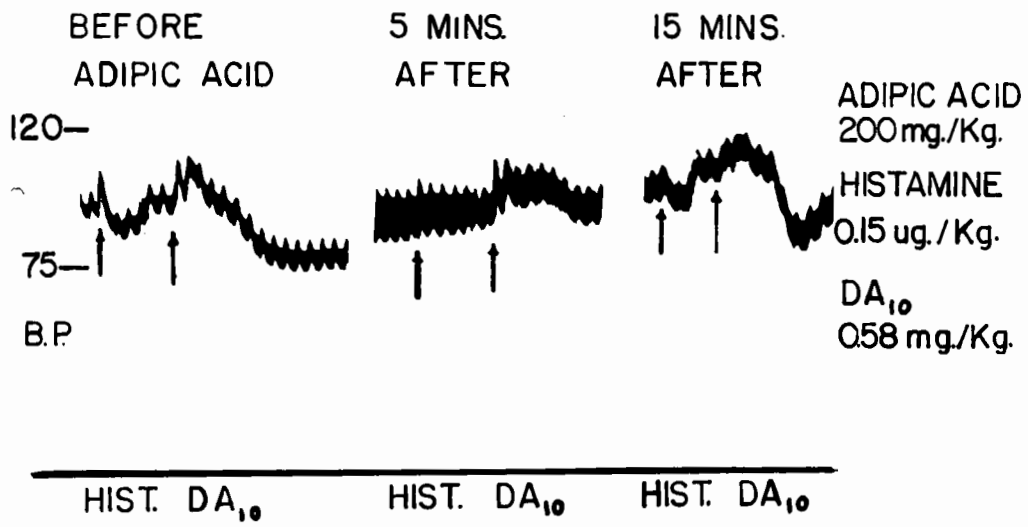


Figure 18

The inhibitory effect of adipic acid on the hypotensive actions of DA_{10} and histamine. Chloralosed cat.

Table 2. Control Series - Percentage Falls in Blood Pressure with the Histamine Liberator, DA_{10} and Histamine. Corresponding Blood Pressures are Tabulated.

Exp. No.	Wt. Kg.	DA_{10} mg.	Hours 0						1						2						3					
257	3.1	1	30	22	13	19	15	15	19	28	23	26	27	25	30	-	28	37	30	25	33	27	47	29	-	-
258	3.4	2	49	38	-	40	48	50	53	46	-	48	39	48	16	10	10	13	10	10	-	-	10	40	45	26
259	4.0	1.5	46	41	13	32	32	40	35	19	25	26	21	21	13	17	9	6	14	7	8	28	6	5		
260	3.5	1	9	13	14	30	26	17	14	5	9	8	-	10	4	5	6	6	8	-	11	13	10	8		
261	3.0	2.5	36	36	28	23	26	33	45	42	35	29	-	30												
262	3.5	2	29	23	22	16																				
265	2.7	1	46	50	31	22	29	30	24	17	39	38	19	11	9	12	15	26	18	12	17	22	14	28	15	26
266	3.0	1.2	46	20	24	20	16	10	12	8	16	13	7	15	13	13										
Averages			32	30	21	25	28	28	29	24	25	27	23	23	14	12	14	18	16	13	17	16	18	22	30	26
Stand. Devs.			14	12	8	8	10	13	14	14	10	13	10	12	8	4	8	10	7	7	9	9	15	13	15	0
Histamine																										
257	3.1	0.3µg	17	22	19	20	28	17	16	16	20	19	25	29	33	-	21	32	29	29	33	36	36			
258	3.4	0.3	-	31	-	26	16	15	19	21	-	22	25	22	26	26	24	26	30	28	--	-	16	12	7	13
259	4.0	1.0	26	15	10	18	9	8	7	4	4	10	16	12	9	15	16	15	15	12	8	12	12			
260	3.5	0.5	30	22	19	18	19	17	21	22	17	13	-	22	26	25	25	24	26	-	28	22	26			
261	3.0	0.5	8	10	11	18	10	21	-	14	20	13														
262	3.5	0.3	9	9	12																					
265	2.7	1.0	26	24	24	24	29	26	23	24	26	29	29	31	29	26	28	37	33	29	27	29	-	27	29	-
266	3.0	0.5	35	36	32	38	40	38	45	26	30	30	29	28	33	-										
Averages			21	21	18	23	19	20	22	24	19	19	25	24	26	23	23	26	26	24	24	25	22	20	18	13
Stand. Devs.			9	9	7	67	11	9	10	8	8	7	5	6	8	4	5	8	6	7	10	9	9	8	10	0
Blood Pressure																										
257	3.1		180	180	180	170	164	158	140	128	120	126	132	130	134	-	134	124	138	134	134	134	132	142		
258	3.4		122	132	-	126	126	124	120	120	-	124	128	130	142	140	140	150	144	144	-	-	150	154	152	150
259	4.0		126	122	128	128	124	114	98	98	104	108	128	134	134	128	132	128	140	142	140	158	152			
260	3.5		140	140	140	132	114	106	112	120	130	132	-	152	144	144	140	142	-	144	150	152	146			
261	3.0		104	104	106	106	116	116	98	100	96	104	-	100												
262	3.5		104	120	134	144																				
265	2.7		110	108	96	90	88	92	100	94	102	104	112	108	112	114	122	124	124	122	128	128	126	128	134	132
266	3.0		86	100	92	108	120	114	148	164	160	170	166	164	172	152										
Averages			121	123	123	125	122	118	116	118	128	124	133	131	133	131	133	133	136	137	138	138	142	144	143	141

700 mg. /Kg. of the acid, neutralized with NaOH, was given.

The mechanism behind the brief inhibitory action of these acids is not understood. In vitro tests showed that adipic acid and DA_{10}, the histamine liberator, do not co-precipitate. Also, when the two substances were injected together into cats the effect was the same as if DA_{10} had been given alone.

(2). Versene

Versene (ethylene diamine tetracetic acid) was shown by Rocha e Silva (1952) to prevent the activation of anaphylatoxin in rabbits blood by agar. Although versene is able to render the blood incoagluable by virtue of its calcium removing action, the absence of calcium in the blood does not prevent anaphylatoxin formation.

In two experiments on cats, di-sodium versenate (Bersworth Chem. Co., Framingham, Mass.) was found to have no more than a brief pressor action in doses up to 15 mg/Kg. (One animal died when given 18 mg/Kg.) Versene did not interfere at any time with responses to either DA_{10} or histamine. In one of the experiments a slight potentiation of the DA_{10} effect occurred.

(3). Suramin (Germanin, Bayer 205)

Guimaraes and Lourie (1951) have recently conducted tests on the inhibition of some pharmacological actions of pentamidine by suramin. Earlier, MacIntosh and Paton (1949) had attributed many of less comfortable pharmacological actions of pentamidine, a drug used against sleeping sickness, to the release of histamine. Lourie found that suramin, also a drug used in the treatment of sleeping sickness, could be injected with pentamidine and prevent the latter from releasing histamine or causing a fall in arterial pressure.

Guimaraes and Lourie were of the opinion that suramin and pen-

tamidine given together would not cause chemotherapeutic interference. Even though suramin and pentamidine form precipitates when mixed, this same precipitate was believed to prevent the impact of high concentrations of drug on vital centers immediately after the injection. A slow dissolution of the precipitate would probably not evoke a release of histamine in dangerous concentrations - it is not known how much the amount of histamine release would be modified.

The polybasic acid, suramin, well-known for treating trypanosome affections, was found to have an anti-liberator action comparable to heparin. Like heparin, suramin coprecipitated with DA_{10} (see Figure 19) and this action was probably the reason for smaller hypotensive effects of DA_{10} when it was given to animals just after the administration of suramin. Figure 20 shows the typical effect obtained for large doses of suramin. The short duration of the effect seems to be much like the one caused by adipic acid.

It is interesting to note that both heparin and suramin have strong affinities for and are able to neutralize haemolytic complement (Jorpes, 1946). However, it is not known if DA_{10} or other liberators act by displacing or influencing complement in any other way.

(4) Phenylpropane derivatives.

By means of a cooperative arrangement with Charles E. Frosst and Co. Montreal, Dr. Irving Levi of that firm was able to provide us with a number of compounds which were hoped to inhibit the release of histamine.

I am indebted to Dr. Levi for the following paragraphs which serve to introduce this section.

"My reasons for suggesting this particular class of compounds (ie. phenylpropane derivatives) are based on the following. Paton (1951)

Mixtures of Suramin and DA_{10} in distilled water.
DA_{10} concentration constant (1%)
Suramin added in increasing concentration.

Tubes	Mixtures						Results
1	1 ml.	DA_{10}	+	1 ml.	Suramin	1/100.000	Clear
2	"	"	"	"	"	1/20.000	Faint opalescence (no precipitate)
3	"	"	"	"	"	1/2000	Dense opalescence (no precipitate)
4	"	"	"	"	"	1/100	White precipitate
5	"	"	"	"	"	1/50	Dense and floculent precipitate
6	"	"	"	"	"	1/25	Faint opalescence
7	"	"	"	"	"	1/10	Clear
8	"	"	"	"	"	1/5	Clear

Figure 19

The <u>in vitro</u> action of suramin on a histamine liberator.

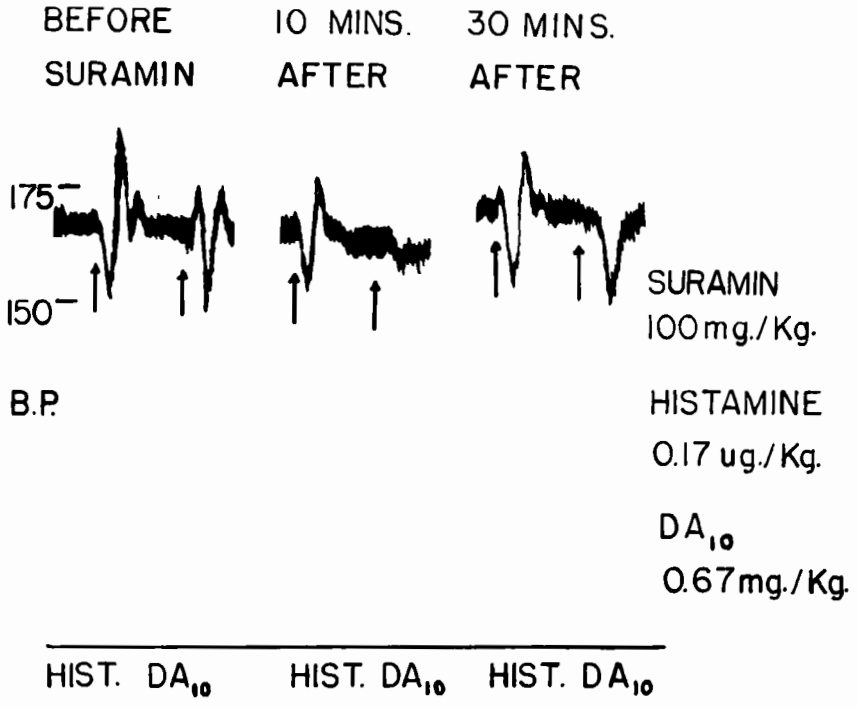
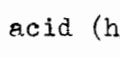


Figure 20

The inhibitory effect of suramin on the hypotensive action of DA_{10} . Chloralosed cat.

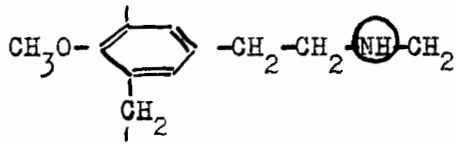
points out that since compound 48/80 releases 10-30 molecules of histamine for each basic unit of its molecule "the mechanism of histamine release by compound 48/80 and presumably by all the other liberators, which in other respects it so closely resembles, must therefore be one by which the liberator can cause the release of considerably more than its own molecular equivalent of histamine". Such a mechanism could conceivably be found in enzyme activation. The reverse mechanism, ie. enzyme inhibition, could then be used to explain the method whereby substances are able to inhibit the release of histamine.

" Rocha e Silva (1946) concluded that the discharge of histamine (and heparin) from the liver of an anaphylaxis-like condition produced in dogs, constitutes the last step in a complex chain of reactions, namely, the probably activation of a proteolytic enzyme (trypsin), followed by the release of histamine (and heparin) by the activated trypsin.

"Neurath, et al(1949) have shown that, of many compounds tested, phenylpropionic acid (hydrocinnamic acid)  was the best inhibitor of a-chymotrypsin. Although a-chymotrypsin is the pancreatic esterase-proteinase and does not liberate histamine, I nevertheless considered it significant that the phenylpropane unit should act as such a strong inhibitor of this enzyme. It was also considered significant that Barnard and Laidler (1952) in a study of the a-chymotrypsin-hydrocinnamic ester system put forth the hypothesis that the active site on the enzyme to which the hydrocinnamic ester attaches itself, consists of a $-COO^-$ group and an $\equiv NH$, the latter belonging to the imidazole ring in a histidine residue.

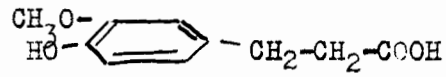
"Compounds containing the phenylpropane unit were therefore thought worthy of consideration with regard to their ability to modify or inhibit

the release of histamine in the living cell. Since each unit of the polymer 48/80 could be considered as a phenylpropane unit in which a nitrogen atom was inserted between two carbon atoms in the side chain, it was thought that similar compounds which did not have the nitrogen atom might act in the opposite manner to that of 48/80, ie. inhibit the release of histamine."



Subsequently, the compounds sent to us from Frosst's represented a few of a large series of naturally occurring phenylpropane compounds and derivatives isolated from lignin.

Following a number of reproducible threshold responses to DA_{10} the compounds were administered, usually in saline, to the cat by slow injection. The results are recorded below in summary form:

1. Hydroferulic acid 

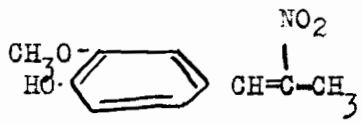
(a) Dose 10 mg/Kg. The response to DA_{10} (1.5 mg) was almost abolished for about 1.5 hours. The response to histamine (1µg.) was unchanged. The blood pressure rose slowly after the injection from 120 to 180 mm. of Hg. The response to DA_{10} returned to normal about 2 hours after the acid was given but the cat died of unknown causes soon after.

(b) Dose 10 mg./Kg. The response to DA_{10} (1.5 mg.) was abolished once only immediately after the acid was given. Histamine responses were constant. The blood pressure rose from 100 to 150 mm. of Hg over a 10 minute period but remained irregular even after the vagi were sectioned.

(c) Dose 16 mg./Kg. (in a 1.3% solution of $NaHCO_3$, pH 8.) There were no changes in the response to DA_{10}, histamine, or in the blood

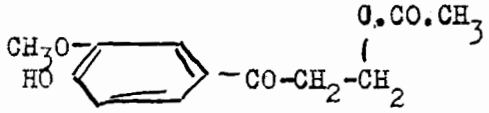
pressure.

(d) Dose 12 mg./Kg. The only change was a slow rise in the blood pressure.

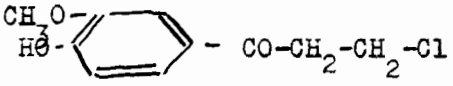
2. 1-Guaicyl, 2-Me, 2-nitro ethylene 

(a) Dose 10 mg./Kg. (in 1.3% $NaHCO_3$). An immediate short lasting pressor effect was seen but there was little effect on the responses to DA_{10} or histamine. A second dose of 20 mg./Kg. caused an increase in the histamine threshold of 20 times (20 µg.) but the response to DA_{10} was not changed. The anti-histaminic effect wore off in about one hour.

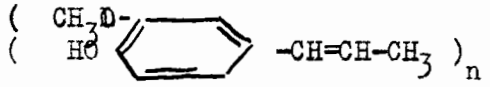
(b) Dose 5 mg./Kg. An immediate short lasting pressor effect was obtained on injection. The threshold of histamine was raised about 4 times the normal value but the DA_{10} response was unchanged. A further 10 mg./Kg. had no greater effect.

3. B-acetoxy propio vanillone 

Dose 9 mg./Kg. (in 1.3% $NaHCO_3$) caused a sharp transient rise in the blood pressure but there were no other changes. A second injection of the compound (15 mg./Kg.) had no greater effect.

4. B-Chloro propio vanillone 

Dose 17 mg./Kg. A sharp immediate rise in the blood pressure was the only significant effect recorded.

5. Polyisoeugenol 

(a) Dose 11 mg./Kg. (in 10 ml. 50% ethyl alcohol). A slight modification of the DA_{10} response alone lasted for about 30 minutes. There was no change in the blood pressure.

(b) Dose - a dose of 20 mg./Kg. killed the cat almost immediately. Solid particles of the substance were noticed within the injection cannula and these may have been at least partly responsible. Since the compound was water insoluble and since most solvents have effects of their own, tests were discontinued.

Most of the drugs tested showed only a slight and fleeting effect, if at all, on the histamine releasing action of DA_{10}. Since the average effect of DA_{10} was usually equal to only a few µg. of histamine, a complete abolition of this small event ought to have been obtained if the substance tested held any promise as an inhibitor.

The insolubility of polyisoeugenol in aqueous media prohibited an accurate estimation of its properties as a histamine-release- inhibitor.

(5.) Alcohol

Our reasons for using ethyl alcohol as a possible inhibitor for the effects of DA_{10} were:

1. To serve as a control for such injections as may require alcohol as a solvent.
2. To attempt inhibition with alcohol itself because it is known that alcoholic liquors frequently produce relief in asthma (but not other allergic conditions). (Feinberg, 1949).
3. Gray, et al, (1951) found that ethyl alcohol was able to abolish the anaphylactic response of the guinea pig ileum to antigen.

In three attempts with diluted alcohol, no reduction of the DA_{10} effect could be obtained. The maximum dose given was 18 ml. of 10% ethyl alcohol per Kg. In one animal 25 ml./Kg. had a fatal result.

(6) Sodium Citrate

There were again several reasons for testing sodium citrate for an inhibitory action against histamine liberators.

(a) Sodium citrate was used in the blood pressure cannula in all experiments. Although the possibility for a large backflow into the animal was slight, because of the air pressure-manometer-recorder, small amounts of citrate may have entered the blood stream.

(b) Rocha e Silva (1952) mentions that citrate has a striking inhibitory effect on anaphylatoxin formation and says it also decreases the amount of histamine released from the dog's liver in anaphylaxis.

(c) McIntyre, Roth and Richards (1949) found that citrate (and oxalate) inhibited the release of histamine from rabbit cells by peptone or antigen.

By employing the usual methods, two cats were given 760 and 166 mg./Kg. of sodium citrate as a 3.8% solution. No change was observed in the responses to either histamine or the histamine liberator following this treatment.

(7.) Typhoid-Paratyphoid (A and B). Vaccine (Parke Davis & Co. Ltd.)

In order to substantiate earlier indications that TAB had no effect on the action of histamine releasing compounds, observations were made on two more cats, tested using the present technique. Rectal thermometers indicated that the vaccine exerted its usual hyperthermic effect, but during almost 4 hours after the injection of TAB (1/2 ml. intravenously) no change in the response to the liberator (ME_2DA_8) was observed.

(8) Acetyl Salicylic Acid (A.S.A.)

Trethewie (1951) had found that sodium salicylate and A.S.A. inhibited the release of histamine from 3 sensitized guinea pig lungs and Gray and associates (1951) confirmed these results for sodium salicylate. When Acetyl Salicylic Acid (A.S.A.), 100 mg./Kg. was hydrolyzed and neutralized with sodium hydroxide and injected into two cats, no inhibitory effect on the threshold responses to ME_2DA_8 was observed over a period of two hours. The insolubility of A.S.A. in saline at a neutral pH prohibited its injection as the acid.

(9) Soya Bean Trypsin Inhibitor (S.B.I.)

In one test with S.B.I. the fall caused by the histamine liberator waned almost to zero 2 hours after the injection of 25 mg./Kg. of S.B.I. However, when the dose of liberator was increased so that threshold responses reappeared, 80 mg./Kg. of S.B.I. had absolutely no inhibitory action. This casts doubt on the possibility that S.B.I. caused the initial waning of the falls occurring after the liberator. In a second experiment after the injection of S.B.I., only 3 responses could be obtained before the animal died. Although the responses were typical no conclusion can be drawn from this experiment. Because of the expense of S.B.I. and other trypsin inhibitors it was decided to forego further experiments of this kind. However, our two trials agreed with the experiments of Scroggie, Jaques and Rocha e Silva (1947) who also could notattenuate canine peptone shock with 20 mg./Kg. doses of S.B.I.

Besides this, one property of S.B.I. is to combine almost instantaneously with trypsin to form an irreversible inactive compound (Northrop, Kunitz and Heriott, (1948). If this occurred with a trypsin-like enzyme supposedly acting in our experiment after its activation by the histamine liberator, ME_2DA_{10}, one would expect that the responses

to the liberator would be nullified immediately after S.B.I. and then slowly return to normal. Since the molecular weight of S.B.I. also prohibits its dispersion from the blood stream after injection, S.B.I. would not be able to inhibit the histamine liberating action of ME_2DA_{10} if that reaction occurred outside of the circulatory system.

Other tests on the S.B.I. used here (personal communication from A. L. Grossberg) indicated that it possessed full enzyme inhibiting activity. Grossberg (1953) showed that inhibition of release from liver cell particles by the histamine liberator, propamidine, required 1000 times more S.B.I. than did inhibition of similar release by crystalline trypsin.

(10) Anoxia

In a previous chapter it was noted that in experiments on the dog where the liver was momentarily excluded from the circulation, a degree of anoxia may have occurred. At that time it was hoped that such anoxia which lasted only a few minutes would not prevent the normal release of liver histamine. Now, we believed that a longer period of anoxia might alter this release by impairing the metabolic processes of the liver. In "Experimental Surgery" (Markowitz, 1937) the development of anaemic necrosis of the liver following ligature of the hepatic artery is described. This necrosis proved fatal in 15 to 20 hours.

In our experiments we therefore determined the threshold values for DA_{10} given both by the femoral and by the portal vein and then clamped off the hepatic artery so that it no longer supplied the liver with oxygen. After 4 and 3.5 hours of liver anoxia in two dogs threshold doses had changed very little. In two other dogs with uninterrupted arterial supplies, there was also no decrease in the responses to DA_{10} by either route. Threshold responses to histamine were unchanged by

this treatment.

(11) Neoantergan and Atropine

Parrot and Burstein (1950) demonstrated that while peptone shock could not be attenuated with injections of atropine or phenergan (an antihistamine) alone, it was greatly diminished following injections of both these agents (Burstein and Parrot (1949) had already shown that atropine itself was a histamine releasing agent.)

In the course of our tests we attempted attenuation of threshold effects of histamine liberators by atropine and neoantergan. Earlier, in a few tests where neoantergan was given alone to abolish the effects of methylated liberators, inhibition did not always occur. On the contrary an increased response was sometimes observed (see Figure 21 B). Now, with atropine alone, potentiating effects were also usually seen (Figure 21 C); and in combination with neoantergan one could not guess which way the deflection would be, although a definite change always took place.

Parrot and Burstein did not attempt to explain why the combination of atropine and neoantergan caused attenuation of peptone shock while either alone had little effect. Similarly, our results were somewhat puzzling, and when seen together (Table 3) the confusion increased. We have already shown that neoantergan alone decreased the hypotensive action of 48/80 (Figure 10), but why should two histamine liberators such as atropine and neoantergan (Pellerat and Murat, 1946) sometimes inhibit the histamine releasing response and sometimes increase it?

The full realization of what was happening did not occur to us until experiments were begun with protamine and the reader is asked to follow up this investigation in that section.

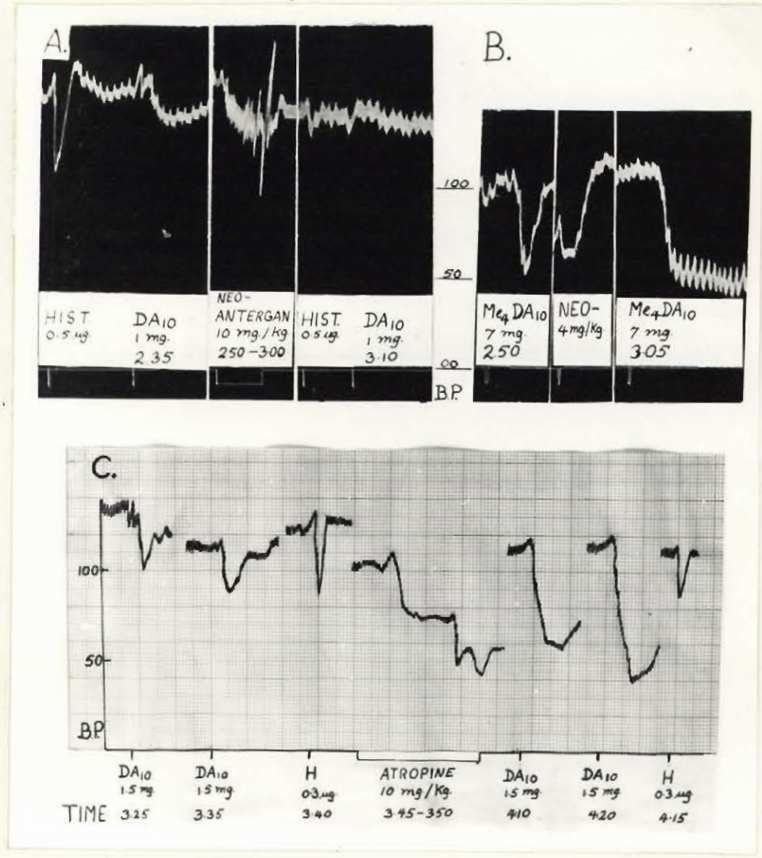


Figure 21

A. The inhibitory effect of Neoantergan on the hypotensive action of DA_{10}. Chloralosed cat.

B. The potentiating effect of Neoantergan on the hypotensive action of Me_4DA_{10}. Chloralosed cat.

C. The potentiating effect of Atropine on the hypotensive action of DA_{10}. (Tracing of original record) Chloralosed cat.

Table 3

Attenuation and Potentiation of the Blood Pressure Response to Histamine Liberators, with Neoantergan and Atropine. (Examples)

Exp. No.	Dose of Liberator and Histamine	% Blood Pressure Falls to Liberator or Histamine Given every ten Minutes									Net Change after influence
166	ME_4DA_{10} 5.5 mg.	7	8	11	Neoantergan	12	Neoantergan	12	Neoantergan	16	increase
	ME_4DA_8 5.0 mg.	5	5	8	0.75 mg./Kg.	18	1.5 mg./Kg.	18	3.5 mg./Kg.	27	increase
	ME_4DA_6 4.5 mg.	23	19	43		40		40		50	--------
	Histamine .5 µg.	22	25	33		15		8		00	decrease
175	DA_{10} 0.8 mg.	3	6	6	Neoantergan	0.75	3	4			decrease
	Histamine 0.2 µg	8	4	10	4 mg./Kg.	3	0	0			decrease
255	DA_{10} 1.5 mg.	21	22	23	Atropine	49	71	51			increase
	Histamine 0.3 µg.	19		28	10 mg./Kg.	18					--------
257	DA_{10} 1 mg.	47	32		Neoantergan and Atropine	20	18.3				decrease
	Histamine 0.3 µg.	36	36		9 mg./Kg. of each	00	00				decrease
258	DA_{10} 2 mg.	26	42		Atropine	54	55		Neoantergan	0 23 25	incr. decr.
	Histamine 0.3 µg.	8	12		10 mg./Kg.	0	0		10 mg./Kg.	0 0 5	decr. ----

(12) Protamine

Because of the intense combining properties of heparin and protamines (Jaques, 1943), and the presence of heparin in conditions where histamine was also released, it was only natural to test protamine for its inhibitory action against histamine releasers.

Two experiments sufficed to make it clear that protamine, far from interfering with the release of histamine, in fact appeared to potentiate the release much as neoantergan and atropine did when they were given just before other histamine-releasing agents.

At this point the suspicion that protamine was itself a histamine releaser set off a number of investigations that eventually provided evidence for a new aspect of the phenomenon of histamine release. This material appears in a later section.

(13) Methylated Aliphatic Diamines

These substances were investigated to provide information of the comparative histamine releasing activities of methylated and non-methylated straight chain diamines. It was a simple matter to show that mono- or bis- dimethylation of the compound 1,10, diamino decane (DA_{10}) would decrease its histamine releasing action. However, as these methylated compounds were also examined for other effects on several species of animals, the results are presented together in the next section of this chapter.

(14) Splenin A and Cortisone

Figure 1 shows schemes of two connected mechanisms dealing with the release of histamine and heparin. The scheme on the right hand side was derived from the papers of Ungar (1951).

Ungar has presented evidence that the "rate of inactivation of fibrinolysin, by its inhibitor antifibrinolysin, is accelerated by hormones from the pituitary gland and the adrenal cortex. The action

appears to be mediated by the spleen since it is absent in splenectomized animals and can be reproduced by a substance in the spleen, Splenin A. In contrast to this accelerating effect of Splenin A, a retarding influence was shown by another substance found in the spleen and bone marrow -- Splenin B".

By cautiously accepting that the final liberator of histamine may be this enzyme designated as fibrinolysin, the substances Splenin A and B became very pertinent to our studies. With a small supply of Splenin A, our first experiment was fortunately a successful one (see Figures 22 and 23).

When 125 units were injected into a 3.75 Kg. cat, no effect appeared until about half an hour had elapsed. Suddenly almost complete inhibition was obtained 100 minutes after the injection, but this disappeared almost as quickly. 375 units more of Splenin A then caused a second waning of the response to the liberator and with this large dose the latent period was considerably shorter. The effects are clearly shown in Figure 23. The DA_{10} effect was abolished at the 100 minute mark and full activity has been regained at 200 minutes. Splenin A did not appear to have any effect on the action of histamine.

One unit of Splenin A was defined by Ungar as that amount of material which reduced the inflammatory passive Arthus reaction in guinea pigs by 50% as measured by the swelling of the ankle joint. The reaction was caused by injecting the ankle joints of guinea pigs with egg albumin one hour after the same animals had received intracardiac injections of anti-egg albumin rabbit serum. (Ungar, Damgaard and Weinstein, 1951).

When the experiment was repeated, again the inhibitory phase took place about the same time and remained low for the period between 1 1/2 and 3 hours. The following table gives a summary of the two runs. Blank spaces indicate that two values were not obtainable to be averaged.

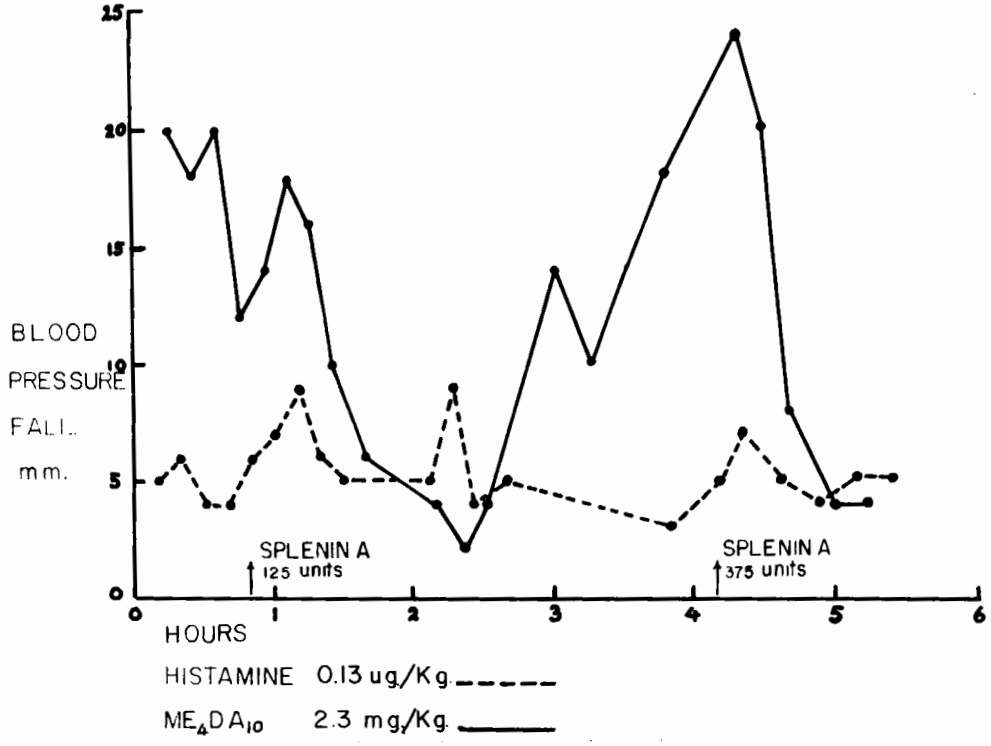
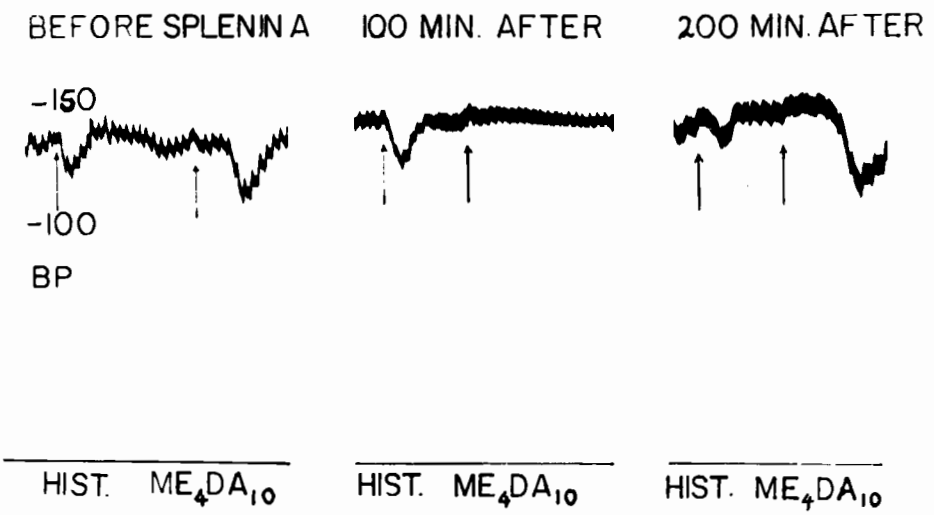


Figure 22

The effect of Splenin A on the hypotensive action of Me_4DA_{10}. Chloralosed cat, 3.75 Kg. See Figure 23 and text for further details.



HISTAMINE 0·13 ug./Kg.
ME_4DA_{10} (1.10-BIS DIMETHYLAMINO DECANE DI HCL) 2·3 mg./Kg.

Figure 23

The effect of Splenin A on the hypotensive action of Me_4DA_{10} (see Figure 22).

Table 4 % Falls in Blood Pressure to DA_{10} and Histamine after Splenin A

Hours	0				1							2				3				
DA_{10}	15	13	14	11	-	15	10	8	7	-	-	1	4	9	3	-	5	5	9	16	20
Hist.	16	17	17	19	-	17	19	-	16	-	-	10	14	9	7	-	-	-	-	9	-

Splenin A 36 units / Kg.

The water soluble preparation of Splenin A which we had been using was now unobtainable from Dr. Ungar, but we were provided with a good amount of Splenin A dissolved in oil. We first attempted to inject this intravenously, as the water soluble preparation had been given, in the form of an egg albumen emulsion, but the cat died after having received only 4 ml. of the 10% egg white emulsion containing 1 ml. (5000) units of Splenin A.

Soon after this experiment there appeared a report that egg white was a strong histamine releasing agent (Schachter, and Talesnik, 1952). This provided a good reason for not injecting any more egg white emulsions.

When acacia was used for suspending the oily preparation of Splenin A, 300 units/Kg. given intravenously caused no inhibition of the histamine liberator effect over a period of two hours.

400 units/Kg. of Splenin A given as an emulsion with Tween 20 also failed to produce an effect over 2 1/2 hours. Tween 20. itself had no hypotensive action in doses equivalent to that given with the emulsion (see Goth et al, 1951).

Finally two cats were given 400 units/Kg. of the oily preparation intramuscularly. In one animal the response to the liberator, ME_2DA_8 was abolished after one hour and in the other animal the abolition occurred

more slowly reaching a maximum between 2 and 3 hours. In only the latter test was there any suggestion of a return to normal deflections caused by the liberator. The duration of the effect may have been due to the size of injection and/or a protracted absorption time from the oil.

(About the 4 hour mark after the intramuscular injection clots had to be washed free from the pressure cannulae. The very next injection of ME_2DA_8 to both cats gave rise to extremely large responses. Subsequent injections of the liberator caused successively smaller responses. In many other experiments this phenomenon had also occurred.

It was not our plan to elucidate the nature of this response, but as a matter of interest we found that fresh cat serum mixed with the liberator produced a response slightly larger than that caused by the liberator alone. This seemed to indicate that neither Splenin A nor sodium citrate had anything to do with this new factor, evidently produced as a by-product of clot formation. It is suggested that this factor is similar to the profibrinolysin accelerator described by Ratnoff (1953) (see Figure 2).

For other investigations with Splenin A please see page 103.

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The scheme (of Figure 1) shows the similar functions played by Splenin A and cortisone in the hormonal mechanism which takes part in the inactivation of fibrinolysin. By means of the general method it was possible to show the effects of Splenin A against the action of histamine liberators very easily. However, cortisone has a relatively slow action after intramuscular injection and our method with cats could not easily be employed to establish its effect against histamine release.

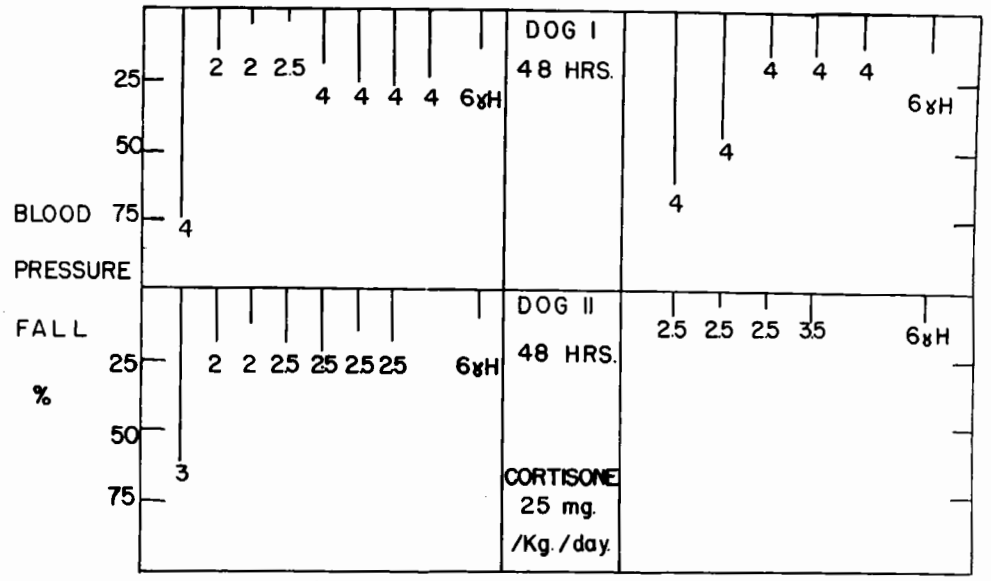
We had already determined that dogs which had received cortisone during an experiment or for several days prior to an experiment responded equally well to histamine liberators before and after cortisone was given. Similarly, Goth, Allman, Merritt, and Holman, (1951) had also been unable to show inhibitory effects of cortisone against first injections of Tween 20.

However, their experiments demonstated the possibility that cortisone interfered with the normal production of histamine in tissues. Where histamine had been released by Tween 20 to cause a profound fall in blood pressure, cortisone given for 2 days to the revived animal usually prevented so great a response to second injections of Tween 20.

The method we adopted to study cortisone's action was a combination of Goth's and the one we had used for cats. Injections of the histamine liberator were given to two animals until constant threshold responses were obtained. One of the animals was then given 24 mg./Kg./day of cortisone for two days. After this time the established threshold doses of the liberator were again given to the two dogs a number of times. Figure 24 shows the results obtained for one of our first sets of dogs.

In the first half of the experiment the two dogs responded almost identically to DA_{10} while its threshold was being established. In the second half the results of two days of cortisone treatment are easily seen. Threshold arterial pressure falls in dogs caused by DA_{10} after cortisone are much less than in the dogs not given cortisone.

In another series similar to that shown in the figure, comparable results were found. The results of this series of six animals, three



EFFECT OF CORTISONE ON THRESHOLD DOSES OF DA_{10} & HISTAMINE

Figure 24

The effect of cortisone on threshold doses of DA_{10} and histamine. The left side of each trial shows the percentage falls in blood pressure obtained while the threshold doses were being established. After 48 hours, during which time Dog #2 received cortisone, the threshold doses of DA_{10} were again given. The effect of cortisone is clearly seen. The response of the blood pressure to histamine, actually given between doses of DA_{10}, has not changed. See text for other details.

of which received cortisone are tabulated in Table 5. It can be readily seen that 2 days of cortisone treatment had definite influence in the amount of histamine released by DA_{10}. The increase in the responses to DA_{10} in the animals which received no cortisone quickly waned after the first two doses. It is suggested that this increase was due to the release of readily available histamine from replenished stores diminished during the first trial. Where cortisone was administered this effect did not occur.

Table 5. The effect of Cortisone on the Release of Histamine (See Figure 25)

A. Animals that received cortisone; 24mg./Kg./day.

Percent Response to DA_{10}

	Before Cortisone			After Cortisone		
No.	1.	2.	3.	1.	2.	3.
Dose	2.5mg.	25mg.	5 mg.	25mg.	25mg.	5 mg.
	18	11	38	7	9	7
	25	13	16	4	3	7
	26	9	13	4	0	7
	24	12	17	-	5	6
	--	--	36	-	-	6
Ave.	23	11	24	5	4	6.6
S.D.	3.5	1.7	10.7	1.3	3.3	.03

Ave. % change after cortisone = -73.2±6.1

Percent Response to Histamine

	Before Cortisone			After Cortisone		
	1.	2.	3.	1.	2.	3.
	6µg.	5µg.	4µg.	6µg.	5µg.	4µg.
	12	13	11	9	20	13
	15	14	11	6	17	9
	11	10	10	-	20	6
	7	12	14	-	21	3
	-	-	-	-	-	-
Ave.	11	12	11	7.8	19	8
S.D.	2.8	1.1	1.5	1.7	2.5	3.6

% change = -2.0±42.4

B. Animals that received no Cortisone

	First Trial			Second Trial		
No.	4.	5.	6.	4.	5.	6.
Dose	4mg.	6mg.	4mg.	4mg.	6mg.	4mg.
	18	12	11	62	43	47
	20	16	8	45	33	18
	14	15	7	14	17	9
	18	17	9	13	14	5
	-	-	9	-	-	3
Ave.	17.6	15	9	33.5	26.6	16.4
S.D.	2.1	1.9	1.3	24	12	16.3

Ave. % change 2 days after first trial. =+81.9 ± 5.8

	First Trial			Second Trial		
	4.	5.	6.	4.	5.	6.
	6µg.	5µg.	6µg.	6µg.	5µg.	6µg.
	8	21	13	8	21	23
	12	28	9	6	20	17
	13	30	8	8	22	16
	12	18	13	6	24	17
	-	-	13	-	-	17
Ave.	11.1	24.3	11.2	7.0	21.7	18
S.D.	1.8	4.8	2.1	0.9	1.57	2.5

% change = +4.6± 33.5

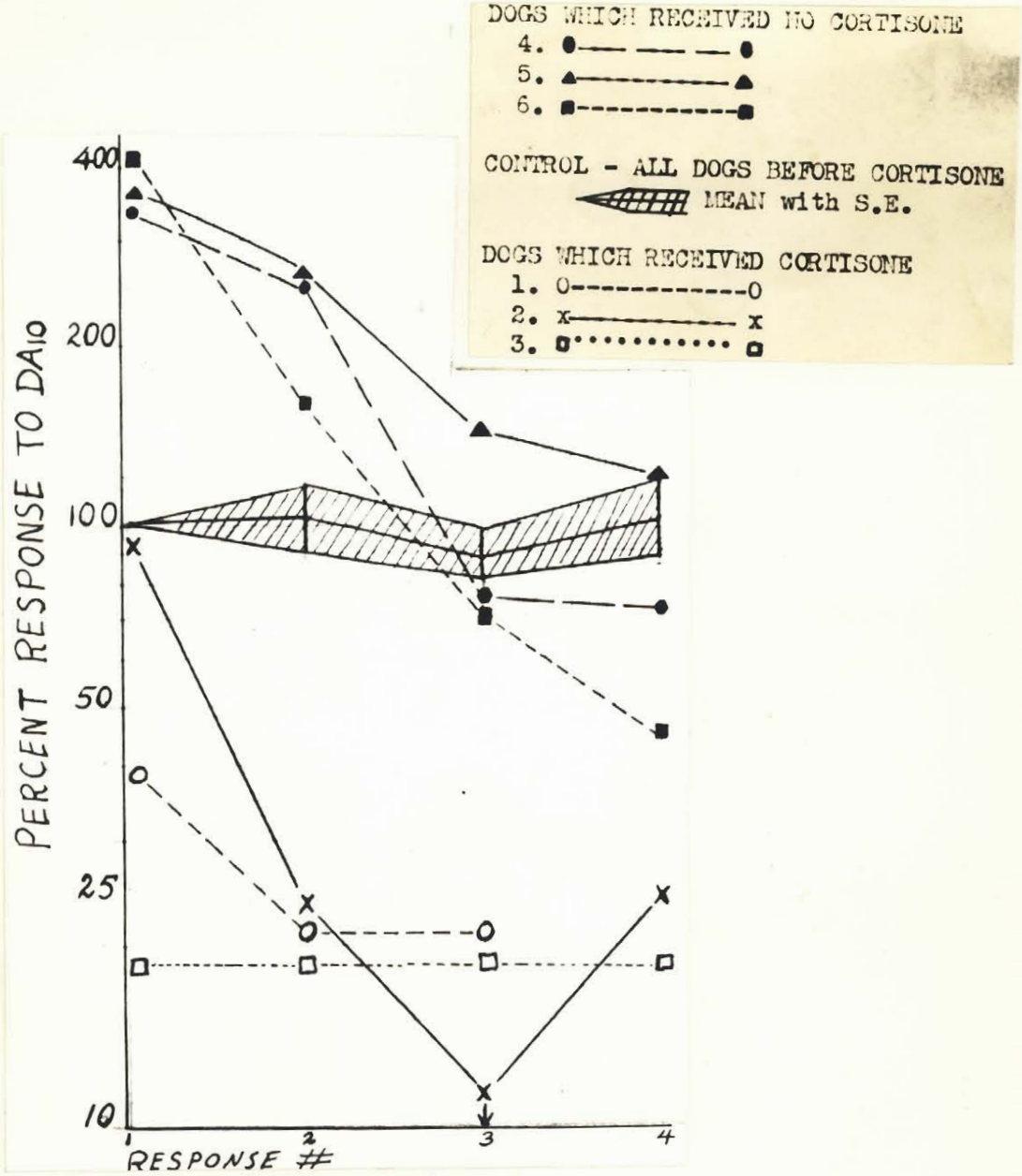


Figure 25

The influence of cortisone on the response of arterial pressure to DA_{10}. To facilitate comparison (since the dogs were of different initial sensitivity) the blood pressure fall in mm. Hg with respect to the first dose of the series was arbitrarily taken as 100. The projection is logarithmic.

As has been mentioned the dose response curve to histamine liberators is, extremely steep and so, seemingly large increases or decreases in the response to a given dose may only represent a dose variation of no more than 10%. Even the 400% increases in the responses shown in Figure 25 probably represent no more than a 20% increase in the dose of histamine liberator. Therefore the effect of cortisone in these experiments may be an extremely small one.

In Table 6 the results of cortisone treatment against larger doses of DA_{10} are seen. In order to deplete histamine stores slowly and also not cause undue hypotension, small doses of the liberator were given at the beginning and a maximum reached after 6 or 8 injections. After cortisone had been given to one of the dogs for two days the same doses were given as before. In both experiments done in this way no great change could be found, in both histamine or DA_{10} values, after one animal had received cortisone. However, one important difference can be seen in the first responses of the second series of doses. The animal that received cortisone responded very poorly to these doses and in this respect the results after large doses of liberator are the same as those after small doses of liberator.

The reason for the large initial response in the second run on the animal which received no cortisone must be connected with a replenished store of histamine whereas cortisone must either (a) prevent the storage of or replenishing the stores of histamine, or (b) deplete the amount of tissue histamine leaving even smaller amounts available for release. A glance at Table 6 will serve to show that, (providing our blood pressure fall records were due to

histamine release) very little histamine has actually been lost during the 2 days between operations. The only difference is that shown by threshold doses of DA_{10}. Therefore, cortisone's action must be focused on histamine that is easily accessible to liberators and readily removed by them. Now, another glance at Figure 24 will serve to show that the large responses caused by DA_{10} in the second operation, where the dog received no cortisone, must be due to histamine that has been newly formed. In the other dog then it is suggested that after a good amount of "readily-available" histamine had been removed by the first series of injections of DA_{10}, cortisone prevented the resynthesis of these stores that were most accessible to the liberator. It is not known whether cortisone prevents production of these stores at the site of release or if cortisone has a distributive action on the total body stores of histamine.

Table 6: Effect of Cortisone on % Falls in Arterial Pressure caused by Histamine and large doses of DA_{10}

Dose of DA_{10} (mg.)		Control dog 17.5 Kg.				**Cortisone-treated dog 20.5Kg.			
		First Op.		Second Op.*		First Op.		Second Op.*	
First Op.	Second Op.	DA_{10}	Hist. 5µg.	DA_{10}	Hist. 5µg.	DA_{10}	Hist. 5µg.	DA_{10}	Hist. 5µg.
3	3	76	14	46	28	29	16	5	20
5	6	77	8	69	32	37	17	20	20
5	10	55	10	41	37	75		36	
7	15	68	15	72	39	87		52	
9	20	58		67		85		67	
11	25	76		71		88		68	

* 2 days after first operation

** 24 mg/kg/day of cortisone (Cortone: Merck).

Applying the same technique in another experiment, doses of ME_2DA_{10} up to 200 mg. were given to two dogs without difficulty. (A total of over 600 mg. could be given within a period of two hours.) When one of these dogs was treated with cortisone only the first few responses to ME_2DA_{10} in the second trial showed any reduction. In dogs not treated with cortisone, responses to the liberator were almost the same in both trials.

When 48/80 was used as the liberator to reduce stores of histamine in three dogs fatal results occurred. After several doses it became increasingly obvious that the dogs were having respiratory trouble. Two autopsies revealed massive haemorrhagic areas and congestion in the lungs. The livers also were engorged. The massive release of histamine in the lungs of these animals probably led to their deaths. The lower potency of the DA_{10} family of liberators probably prevents them from recruiting histamine from the lungs and the action of DA_{10} must only take place where histamine is more easily released - as in the liver.

One last experiment again showed that cortisone probably does not exert its effect by depleting stores of readily available histamine. In two dogs, thresholds for DA_{10} were found both before and after one dog had received 24 mg./Kg. of cortisone for two days. The thresholds were obtained very carefully by giving doses of DA_{10} no greater than was necessary for the smallest blood pressure deflection. In this way practically no histamine would be released. The results for this test are tabulated below.

Table 7: Thresholds to DA_{10} before and after cortisone.

	First trial		Second trial
Dog 1	.24 mg./Kg.	24 mg/Kg./day cortisone i.m.	.24 mg./Kg.
Dog 2	.40 mg./Kg.	control	.30mg./Kg.

As can be seen cortisone had little effect on the action of the liberator. The histamine threshold of .095 µg/Kg. remained constant for both animals. Our results agree reasonably well with the investigations of other investigators. Devitt, Pirozynski and Samuels (1953) found no significant difference in the numbers of mast cells in rats treated with cortisone. (Mast cells have been suggested by Riley and West (1953) to contain histamine as well as heparin.) Baker, Ingle and Li (1951), like Devitt, could also find no change in mast cells of rats treated with cortisone.

On the other hand, Bloom (1952) treated multiple malignant mastocytomas in a dog with cortisone and found that after 9 days they had disappeared. Cavallero and Braccini (1951) and Asboe-Hansen (1952) have obtained a decrease in the number of mast cells usually found in various tissues by treating rats, guinea pigs and humans with ACTH and cortisone. They and Stuart (1951) claim to have observed various morphological changes in the cells after cortisone treatment. On the basis of Devitt's morphological study of the mast cell some doubt is cast on the investigations of these other workers.

Although our experiments with cortisone and Splenin A were made on two different species the scheme of Ungar (Figure 1) is compatible with the observations, and has, in fact, been given added support. We consider that Splenin A is apparently able to retard the rate of antifibrinolysin inactivation and thereby prevent the release of histamine by activated fibrinolysin. It is unfortunate that many more experiments could not have been done with Splenin A, but it is hoped experiments of this kind will be continued to further elucidate the

nature of the hormonal participation in fibrinolytic mechanisms.

As well as adding evidence to the suggestion of Goth, et al, (1951) that cortisone prevents the resynthesis of histamine in tissues where the amine has been released, our experiments with cortisone suggest that only loosely bound and newly formed histamine takes part in liberation processes of a "threshold" nature. Again, it is regrettable that more animals could not be tested for this effect.

Actually our experiments do not show that histamine formation is in any way directly connected with cortisone. It is just as probable, if Ungar's scheme is considered, that cortisone's action is to retard antifibrinolysin's inactivation. If so, the action of fibrinolysin would consequently be prevented by the antifibrinolysin and histamine would not be released. The exhaustion of the fibrinolytic factor by large doses of histamine liberators would just as easily account for the "refractory" phase as would the exhaustion of histamine. And since the stores of histamine in the body appear to be very much greater than are released in any instance of "histamine release", it is more probable that the refractoriness comes about by fibrinolysin depletion. The action of cortisone when given to a dog recovering from "histamine-releaser-shock" would be to extend the period of refractoriness by preventing, via increasing amounts of antifibrinolysin, the action of activated fibrinolysin.

The Action of Cortisone on the Histamine Content of Cats' Skin

In the dog experiments, cortisone had had no apparent effect on the availability of histamine to initial doses of histamine liberator. To test this in other species, the high content of histamine

in the cat's skin suggested an easy means for providing small pieces of tissue to be tested for histamine content before and after cortisone treatment. It was also hoped that this type of experiment might also substantiate the results of the Splenin A experiments which were also done on cats and thus, provide further identification of the hormonal influences on the fibrinolytic system which has been suggested to cause the release of histamine.

Small portions of skin were obtained from cats under ether anesthesia from the inside of one hind leg and from the opposite flank. Histamine values for these samples were then estimated by means of the guinea pig gut preparation. After 3 of these animals had received 25 mg./Kg./day of cortisone for 2 days, second pieces of skin from the other flank and inside hind leg were removed. The following results were obtained.

Skin Histamine before and after Cortisone Treatment (µg./Gm.)

Control Animals

Exp. No.	1st operation	2nd operation	Difference
267	21.6) 21.1) 21.3	25.2) 17.2) 21.2	----
274	25.7) 26.8) 26.21	19.5) 16.0) 17.7	- 35%
290	30.9) 21.0) 25.91	42.8) 46.2) 44.5	+ 172%

Cortisone Treated Animals (25mg./Kg./day)

267	37) 36) 36.5	42.7) 29.5) 36.1	----
274	28.4) 26.4) 27.4	14.7) 18.1) 16.4	- 40%
290	21.0) 15.7) 18.3	32.6) 30.0) 31.3	+ 173%

It is odd that the same type of variation occurred with each set of animals even when one had been receiving cortisone for several days. Because of this and the apparent lack of response of the skin histamine of cats to cortisone, experiments of this kind were discontinued.

E. THE HISTAMINE RELEASING ACTION AND OTHER PHARMACOLOGICAL ASPECTS OF A GROUP OF METHYLATED ALIPHATIC AMINES.

Many investigators have demonstrated the ability of a large number of chemical compounds to liberate histamine in vivo (MacIntosh and Paton, 1949; Reid, 1950; Feldberg and Paton, 1951; Paton, 1951). Such compounds as peptone, the curare alkaloids, licheniformin, stilbamidine and the synthetic polymer 48/80 cause the characteristic hypotensive reaction which begins about 20 seconds after injection. Highly active in this class of substances are the simple aliphatic diamines, diguanidines, diisothioureas and bis-trimethylammonium compounds, provided they have a chain length of from 6 to 12 carbons. (MacIntosh and Paton, 1949). The member of these series become increasingly potent with increasing chain length up to about the C_{10} compounds, and then become gradually weaker as the chain lengthens further. It appears that histamine releasing activity in these compounds is mainly a function of the distance between the terminal basic groups; the nature of the basic groups whether $-NH_2$, $-C(:NH)NH_2$, $-NHC(:NH)NH_2$, $-SC(:NH)NH_2$ or $-N(CH_3)_3^+$, makes less difference.

The purpose of our experiments was to determine whether methylation of the two polar amino groups would alter the various pharmacological activities of aliphatic diamines. The investigation appeared to be justified by the fact that a number of new drugs contain two or more secondary or tertiary amino groups.

Used in all the experiments were the simple primary compound, decamethyldiamine dihydrochloride, and the dihydrochlorides of its bis-mono-methyl and bis-di-methyl derivatives. These will be referred

to as DA_{10}, ME_2DA_{10}, and ME_4DA_{10}. In order to assess the importance of chain length in the series of secondary and teriary diamines, the C_6 and C_8 bis-mono-methyl (and bis-di-methyl) derivatives have also been examined. They will be referred to as ME_2DA_6, ME_2DA_8, ME_4DA_6 and ME_4DA_8.

Most of the methods used have already been described under Routine Procedures or in the previous section. New procedures will be included with other details of the experiments.

Results

1. The Action of Methylated Aliphatic Diamines on the Blood Pressure

Records of the arterial pressure of cats and dogs showed that these compounds act on the circulation exactly like the primary diamines: ie. they produce a sharp fall of pressure which does not begin until 15-25 seconds after the intravenous injection. The latent period of 15-25 seconds is characteristic of the action of histamine liberators: histamine itself acts within a few seconds. The actual latent period of the fall caused by any liberator is constant for any one animal. The latent period does not appear to be affected by the chemical nature of the liberator.

The duration of the depressor response caused by small amounts of any of the methylated compounds was usually about the same as, or slightly longer than that obtained for doses of histamine that produced an equal fall of arterial pressure. (Figure 26). With progressive increase in dose, the prolongation of the depressor effect was more striking for the methylated compounds than for histamine itself. With very large, but sub-lethal, doses of a methylated compound, the arterial pressure usually remained low for hours.

When one of the compounds was administered repeatedly in increasing

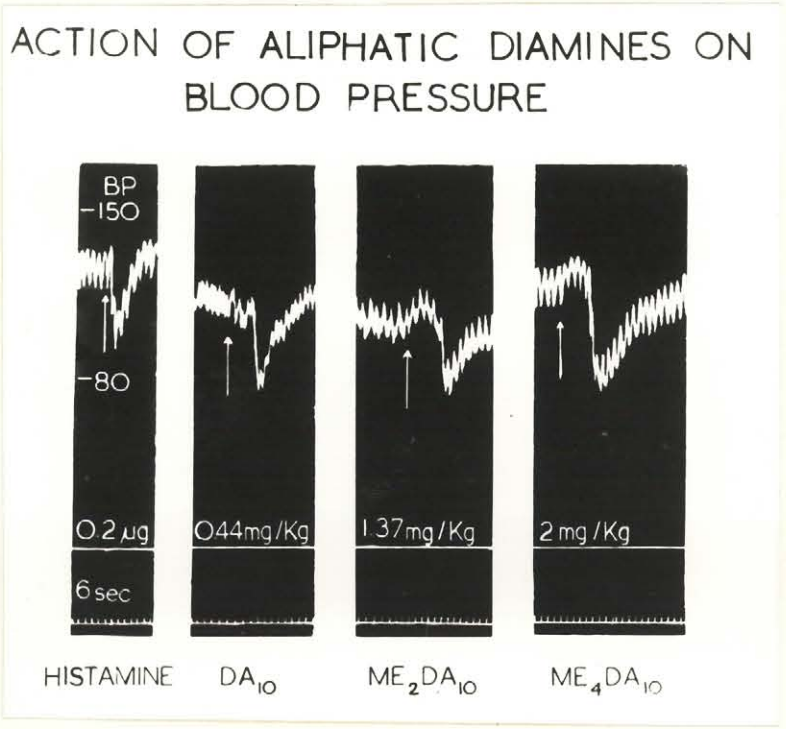


Figure 26

The action of aliphatic diamines on the cat's blood pressure. Approximate threshold responses have been obtained. The latent periods of the response for the aliphatic diamines are obviously longer than that for histamine.

dosage, beginning with a subthreshold dose, the onset of a frank action was usually sudden-often the arterial pressure fell by 30-40 mm. after a dose only 10% larger than a dose that produced no apparent effect. Furthermore, when a subthreshold dose was repeated a few minutes later, it often produced a striking effect. The threshold dose for apparent histamine release could be determined rather accurately by injecting fractional doses at brief intervals. The latent effect of a subthreshold doses wears off within a few minutes (Figure 9) provided the dose is not too large, and when injections are made 10 minutes or more apart, the effect of a threshold dose is reproducible.

When the dose of any of the compounds is not more than twice threshold, the depressor effect is of moderate size and usually wears off within a few minutes at most. Larger doses (eg. 3-4 times normal) have a much more prolonged effect; and still larger doses (eg. 10 times threshold) produce a vascular collapse that is eventually fatal. The circulatory shock produced by any of the compounds is very similar to histamine shock; thus the haemotocrit value is elevated and the heart continues to beat strongly until shortly before death.

In 6 cats, the mean threshold dose in mg./Kg. for DA_{10} and for the methylated compounds were as follows:

DA_{10}	0.29				
ME_2DA_{10}	0.85	ME_2DA_8	0.76	ME_2DA_6	0.44
ME_4DA_{10}	1.86	ME_4DA_8	1.92	ME_4DA_6	2.06

The values of p for difference between these thresholds were as follows:

DA_{10} and ME_2DA_{10}	$0.01 > p > 0.001$
DA_{10} and ME_4DA_{10}	$0.01 > p > 0.001$
ME_2DA_{10} and ME_4DA_{10}	$0.3 > p > 0.2$

ME_2DA_{10} and ME_2DA_6 $0.001 >> p$

ME_2DA_8 and ME_2DA_6 $p = 0.001$ (approx.)

DA_{10} and ME_2DA_6 $p = 0.1$ (approx.)

The p. values between figures for the bis-di-methylated compounds (ME_4-) were all greater than 0.3. The p values between ME_4 - and ME_2 - compounds in the C_6 and C_8 series were also of this order.

We may therefore conclude from these values that:

(a) Bis-di or bis-mono-methylation of the aliphatic diamine DA_{10} significantly reduces the threshold value of histamine releasing activity.

(b) The difference between the threshold values for bis-mono and bis-di-methylated compounds of any chain length is not significant.

(c) The threshold value of bis-mono-methylated diamines increases significantly with decreasing chain length. There is no significant difference in threshold values for bis-di-methylated compounds having different chain lengths.

2. Action of an Antihistamine on the Hypotensive Effect of the Bis-di-Methylated Compounds

In 3 cats the intravenous injection of mepyramine (=neoantergan) maleate promptly reduced the depressor response to ME_4DA_{10}, provided the latter was given in just suprathreshold dosage. In such experiments, the depressor response returned to normal when the effect of the antihistamine drugs were off. Mepyramine likewise reduced the depressor response to such known histamine liberators as DA_{10} and 48/80 when they were given in low dosage. When bigger doses of any of these compounds were used, the response could not be depressed by even very large amounts of mepyramine (up to 12 mg./Kg.): sometimes, indeed, it was increased. The failure of mepyramine to antagonize

completely the depressor effect of the methylated base does not, of course, exclude the possibility that this effect depends entirely on the release of histamine (MacIntosh and Paton (1949).

3. The Action of Methylated Diamines on Isolated Guinea Pig and Rabbit Intestine

The parent compound DA_{10} evoked a slight contraction of the guinea pig gut only when its concentration in the suspension fluid was 10^{-3} or higher; in lower concentrations (down to 10^{-5}) the tone of the gut was unaffected but its sensitivity to both histamine and acetylcholine was greatly reduced. The methylated compounds in similar concentrations likewise depressed the sensitivity of the gut to histamine and acetylcholine; they appeared to lack the weak stimulant action of DA_{10} even in concentrations as high as 10^{-2}. However, in this concentration the spontaneous contractions of the gut were increased slightly. A number of the chemically similar compounds that have been found effective as histamine liberators also possess moderate antihistaminic and atropine-like activity (MacIntosh and Paton, 1949, Paton, 1951).

In the rabbit gut both DA_{10} and ME_4DA_{10} (10^{-3} to 10^{-4}) reduced both the tone of the preparation and the amplitude of the spontaneous contractions. The affect was at least superficially similar to that of adrenalin.

4. The Release of Histamine by Methylated Diamines

After injections of a moderately large dose of ME_4DA_8 (5 mg./Kg. in each of 2 cats and 7 mg./Kg. in one dog) blood samples were taken at 1 and 30 minutes later for the estimation of histamine. Control samples of blood were taken before the injection. In two trials the

histamine values for cat plasma samples taken at 1.1 and 30 minutes were respectively 0.11, 0.22 and 0.06 μg./ml. and 0.45 0./80 and 0.063 μg./ml. One set of dog plasma for the same times was <0.01, 0.95 and <0.01 μg./ml. (Figure 27). In a cat experiment with ME_2DA_8 the corresponding values were <0.010, 0.127 and 0.075μg./ml. In all the experiments mepyramine reduced equally the response of the gut to both the histamine and plasma values and the response to both reappeared pari passu as the effect of the antihistamine wore off. The other methylated diamines were not directly tested for histamine-releasing activity; but since they are so like the tested compounds, both in chemical structure and pharmacological action, it seems safe to conclude that they also belong to the same class of histamine liberators.

5. The Release of Heparin by ME_4DA_8 in the Dog

It has been noticed by other investigators (Waters, Markowitz and Jaques, 1938; Wilander, 1938; MacIntosh and Paton, 1949; and Reid, 1950), working with histamine releasing compounds that the release of histamine in the dog is frequantly accompanied by an increase in the clotting time of the blood. That this phenomenon is due to the release of heparin has been indicated (a) by the ability of the plasma to induce metachromasia in a solution of toluidine blue or a related dye (Jorpes, 1935; Jaques, Mitford and Ricker, 1947) and (b) by the isolation of heparin from the blood as the barium salt (Wilander, 1938; Jaques and Waters, 1941). In several of our dog experiments we noted an increase in clotting times after the injection of a methylated diamine. In one such experiment with ME_4DA_8,

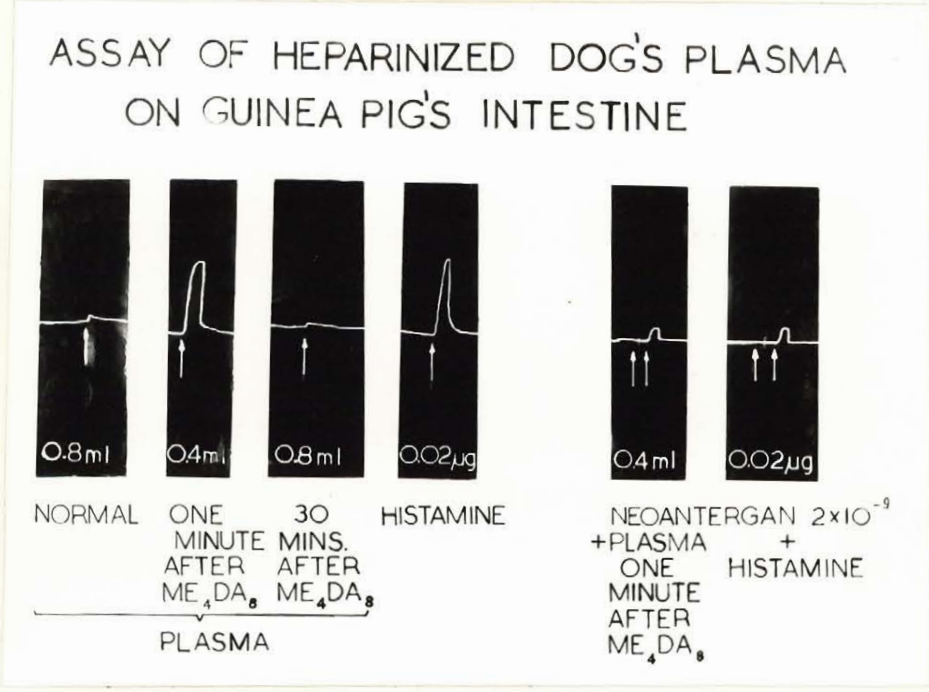


Figure 27

The assay of dog's plasma for histamine after the administration to the dog of Me_4DA_8 (5mg./Kg.). Neoantergan (at the right) was injected into the bath containing the gut 30 seconds before histamine. One ml. of plasma obtained 1 minute after Me_4DA_8 is calculated to contain 0.05 µg. of histamine.

the metachromatic activity of the serum, obtained when a blood sample, obtained 5 minutes after the injection, had eventually clotted, was found to be some 12 times greater than that of serum obtained before the injection, (Figure 28) and was approximately equal to that of a 10^{-5} solution of commercial heparin. The methylated diamines themselves have only a very weak anticoagulant action *in vitro* and do not produce metachromasia. The increased clotting time and metachromatic activity that followed the injection of ME_4DA_8 was therefore presumably due to the release of heparin.

6. The Toxicity of Methylated Aliphatic Amines on Mice

The L.D.50 by intraperitoneal injection in groups of 5 mice was found to be for ME_4DA_3, ME_4DA_{10} and DA_{10} respectively 150, 168 and 145 mg./Kg. mouse. (Figure 29). Thus, in spite of the fact that these compounds differ significantly in respect to their depressor potency in the cat, there is no corresponding difference in their toxicity for the mouse. (On a molar basis the toxicities of the 3 substances agree still more closely). The times elapsing before death was respectively 11, 9, and 6 minutes, the standard errors for these values being 0.72, 0.37 and 1.3. The bis-mono-methylated compound ME_2DA_8 was found to have an L.D.50 of 434 mg./Kg. mouse. (Figure 29).

The first noticeable sign of poisoning was sluggishness. This was followed by general excitability, atoxia, dyspnea and clonic convulsions. Nose scratching was occasionally noticed. Post-mortem, nothing remarkable was observed, except perhaps unusual pulmonary congestion.

Since histamine is known to be more toxic to mice after adrenalectomy (Halpern, 1950) the toxicity of DA_{10} was retested on 4 groups of 4 mice each adrenalectomized 6 or 22 hours previously.

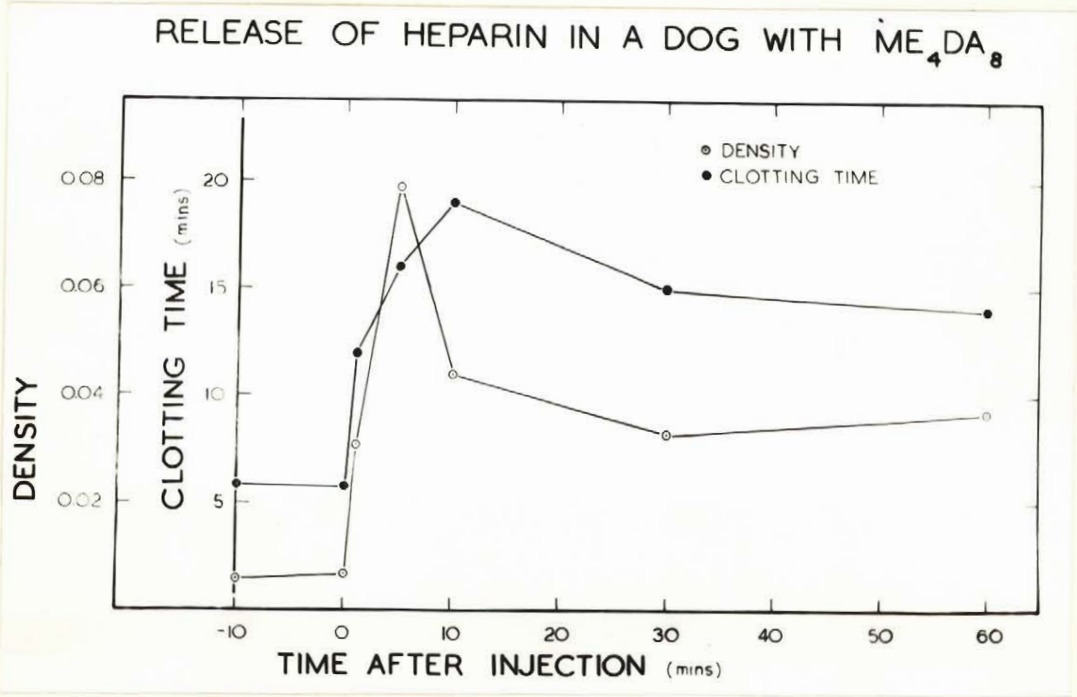


Figure 28

The release of heparin in a dog by the administration of Me_4DA_8 (5mg./Kg.). Density refers to the degree of metachromasia obtained when the serum is added to a solution of the dye Azure A. Clotting times were determined on whole blood by the Lee-White method.

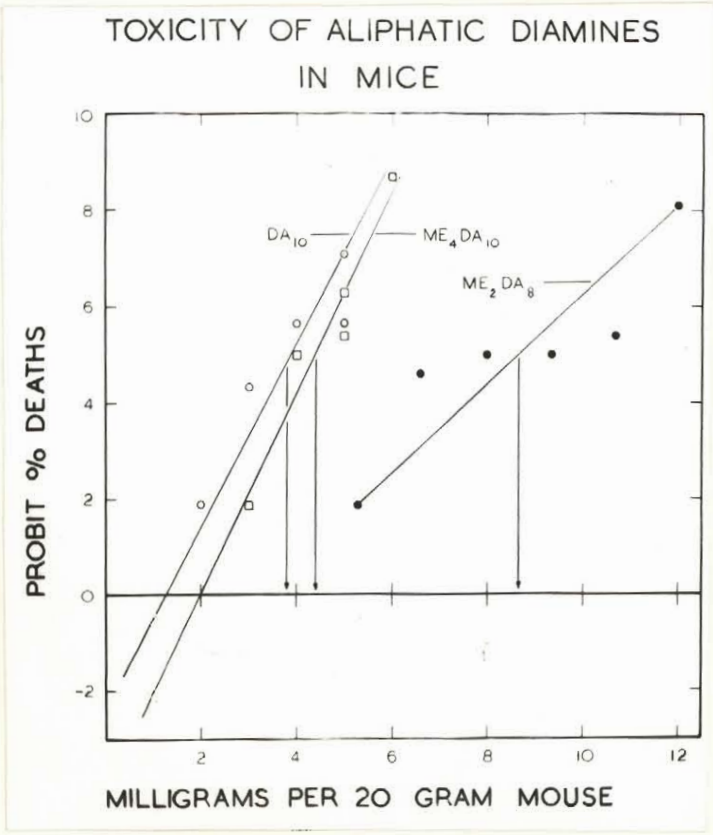


Figure 29

The toxicity of aliphatic amines in mice. The point on the regression line opposite probit value 5 is the L.D. 50. (In the text the L.D. 50 values have been recorded as mg./Kg. mouse.)

Adrenalectomy produced no significant difference in either the lethal dose of DA_{10} or in the average survival time. The symptoms preceding death were the same as for intact animals. Unfortunately the number of mice available at the time prevented us from testing the effects of the methylated compounds on adrenalectomized subjects, but since the methylated compounds are so like the tested compound it is probable that they also would show no significant difference in their lethal effects against adrenalectomized mice.

Our experiments have shown that bis-mono-methylated and bis-di-methylated aliphatic compounds are able to release histamine in intact cats and dogs. The methyl diamines likewise resemble their primary analogues in their ability to release heparin as well as histamine. Further resemblance among these drugs is noted in their heparin releasing action. Qualitatively their action seems to be entirely similar to that of the primary amines. Quantitatively, methylation effects potency but no simple generalization will express the extent or even the direction of the effect.

In the series of primary diamines, MacIntosh and Paton (1949) found a sharp peak for pharmacological activity with the C_{10} and C_{11} compounds. With the methylated substances this was not the case; with the three secondary diamines tested activity varied inversely with the chain lengths; and with the three tertiary diamines it was little affected by the chain length. No doubt an explanation of these differences will eventually be explained in terms of the different positive charge on the terminal basic groups and steric relationship between these groups and negative "receptor" groups at the site of histamine release.

Alles, Ellis and Redeman (1953) have recently carried out an independent study of the methylated alkane diamines. These authors observed no delayed depressor response for either methylated or non-methylated compounds in dogs, rabbits or guinea pigs, whereas this effect was quite evident in our studies on dogs and cats. The claim by Alles, et al, that no delayed response occurs after injections of the methylated compounds is not supported by the records that they submitted. The responses caused by their compounds appear to occur (in the absence of a time scale) at least several seconds later than responses due to injections of histamine. Alles and coworkers also found no evidence for histamine release in their experiments, but this was tested for in guinea pigs only. The marked depressor effects caused by the compounds used were attributed by them to be due to direct effects and not to released histamine. Our results indicate that this interpretation certainly does not apply to cats and dogs and the conclusion reached by MacIntosh and Paton (1949) that such compounds as DA_{10} are histamine releasers has been fully verified in our laboratories.

Besides this, Alles, et al, suggest that MacIntosh and Paton (1949) should have carried out their studies on the most active alkane diamine, the C_{10} compound, for more clearly defined characteristic actions of the series. However Alles and his coworkers, themselves, only tested that one compound as the diamine and did not show that it possessed the most "specific" actions of the series. Perhaps our own results are more reasonable in this respect where various compounds of the methylated series have been used to test different activities. The "specific" activity of such compounds

seems to extend throughout the series.

The pKa values of the ME_4 and ME_2 compounds were found to be very closely grouped around their averages of 10.00 and 10.64 respectively and the value for DA_{10} was 0.57. These values correspond to relative ratios in toxicity of 1, 4.4, and 3.7 (ME_4-,ME_2-, and DA_{10}) whereas their threshold hypotensive strengths were in the ratios of 1, 3 and 6. MacIntosh and Paton, (1949) have shown that the still more basic diquaternary ammonium compounds have even less activity as histamine liberators. However, the possibility that basicity influences the histamine releasing ability of these compounds is reserved until more positive results are obtained.

Whether the intestinal muscle stimulating effect of DA_{10} was due to the histamine releasing action or another effect is not known. Since in all other actions DA_{10} was found to have a potency equal to the methylated compounds, its greater ability to release histamine suggests that it may be responsible for the gut contracting action. However, no other drugs were used to elucidate the nature of this effect and therefore its origin is unknown.

Certain reasons indicate that if histamine was released by the drugs in the toxicity tests it was not responsible for killing the mice. These are:

(1) DA_{10} is six times stronger than the bis-di-methylated derivatives in producing a fall in the blood pressure of the cat, presumably by the release of histamine, whereas the M.L.D. is about the same for these compounds. Also where bis-mono-methylated compounds are several times stronger than bis-di-methylated compounds in eliciting the release phenomena as shown by the drop in blood pressure, one bis-mono-methylated drug tested had an L.D.50 on

mice three times larger than DA_{10} and any of the bis-di-methylated group. This inverse relationship indicates that the two phenomena are probably unrelated and that the toxic effect of the compounds is not due to the release of histamine.

(2) Mice are extremely resistant to histamine. For normal mice Halpern and Wood (1950) have found that the L.D.50 for histamine dihydrochloride, intraperitoneally, is 50 mg. per 20 Gm. mouse. The great difficulty in inducing anaphylaxis in them has been attributed to this cause (Saunders, 1951). A second reason why the mouse would not be greatly affected by anaphylaxis, even if all the histamine was liberated, is that it contains not more than 10 mgs./Kg. of histamine of body weight (Mayer, 1950). It is therefore improbable that such small doses of our compounds would cause death by releasing histamine.

(3) Halpern and Wood (1950) have shown that adrenalectomized mice are more susceptible to histamine than intact mice (L.D.50: 0.50 mg./20 Gm. mouse). That the adrenal glands were not influencing the toxicity of the primary diamine, DA_{10} was ensured by repeating the toxicity test on adrenalectomized animals. It is believed that adrenalectomy would likewise not affect the toxicity of mice to similar compounds of the methylated series.

Mayer (11) has assumed that substances other than histamine are responsible for anaphylaxis in the mouse. This is strongly supported by the fact that anaphylaxis in these rodents is essentially unaffected by some antihistamines. The striking resemblance in the activity exhibited by mice when given a lethal dose of a histamine liberator to that manifested during anaphylactic shock induced by horse serum, (Nelson,

Fox and Freeman, 1950) is probably significant. The possibility is suggested that a substance other than histamine liberated as a by-product of the antigen-antibody or histamine-liberator reaction may be responsible for the features recognized as being common bo both fatal conditions.

In summary, some pharmacological properties of a group of bis-mono-methylated and bis-di-methylated aliphatic diamines have been examined. These compounds have demonstrated their ability to release histamine in the cat, and histamine and heparin in the dog. The methylated C_{10} compound is several times less active than the analogous non-methylated diamine, which has already been investigated, but qualitatively its action is entirely similar. In toxicity tests, no essential difference between the methylated and non-methylated compounds was observed.

F. Studies with protamine and polylysine

In experiments on the release of histamine in animals, we had been attempting to prevent such a release by various methods. It seemed possible that protamine might have this inhibiting effect by virtue of its relation to heparin, with which it intensely combines, and because, in conditions of histamine release in the dog, heparin is also liberated. Chargaff (1938) and Jaques (1943) had shown that protamine combines with heparin to prevent the former's anticoagulent action. Also, many workers (Jaques and Waters, 1944; MacIntosh and Paton, 1949; Reid, 1950; Garcia-Arocha and Ashwin, 1952) have found evidence that when histamine is released in anaphylactic and/or anaphylactoid shock, there is usually a concomitant release of heparin.

After we had established a constant hypotensive response in the arterial pressure caused by the histamine releaser DA_{10}, 30 milligrams of the protamine, clupein, was slowly infused in the animal. To our surprise, subsequent injections of DA_{10} were not inhibited but potentiated (Figure 30). As we continued DA_{10} injections, every 10 minutes, the supranormal effect gradually wore off and responses became normal.

Reviewing earlier experiments, a sudden suspicion that protamine caused the release of histamine became prominent. We had found that subthreshold doses of a known liberator given repeatedly one or two minutes apart may eventually add up and cause the characteristic hypotensive effect (Figure 9). Two releasers, such as DA_{10} and 48/80,

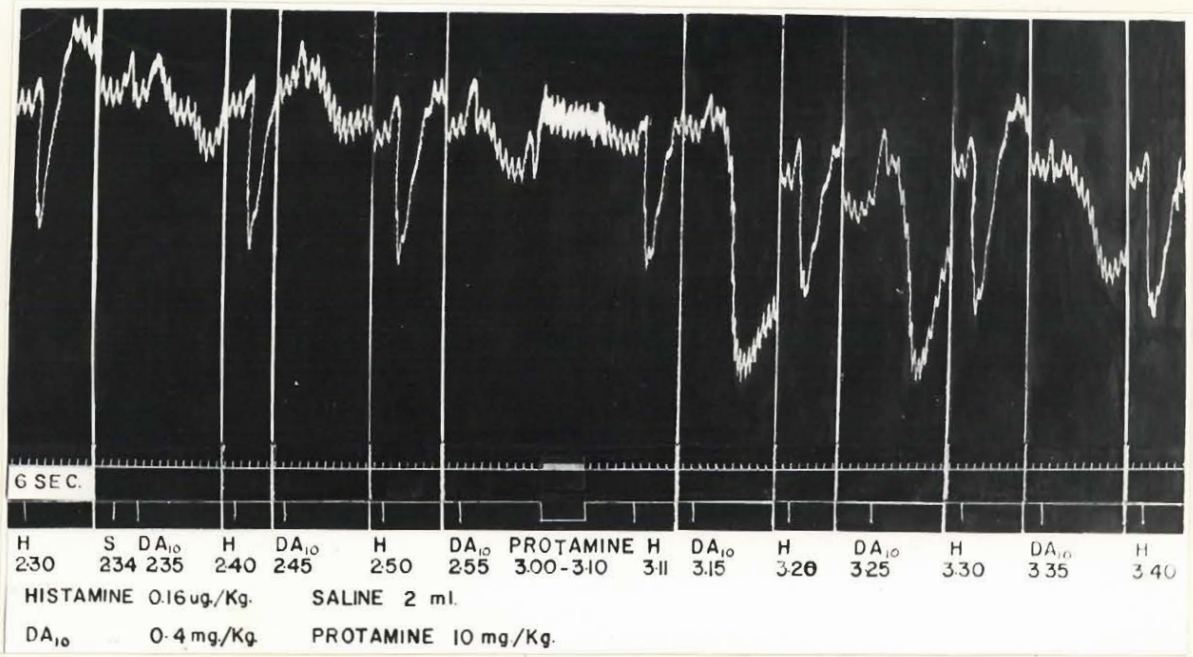


Figure 30

The effect of protamine (clupein) on the hypotensive action on the cat's blood pressure of DA_{10} and histamine. Cat, 3 Kg., chloralose. After recording responses to DA_{10} and histamine every 10 minutes for over an hour, 30 mg. of protamine was slowly injected intravenously. The subsequent responses of the arterial pressure to DA_{10} are considerably greater than those before protamine. The responses to histamine have not been changed by protamine.

given together or alternately a short time apart, are also able to produce a potentiated effect (Figure 7).

In other experiments we had sometimes noticed potentiation of the response to DA_{10} occuring after both atropine and neoantergan (Figure 21). Thus our results complemented those of Schachter (1952) who had shown the ability of atropine to release histamine, and also agreed with the work of Arunlakshana (1951) and Pellerat and Murat (1946) who showed that antihistamines themselves may cause the release of histamine.

From these data, protamine was strongly suspected as being a "histamine releaser".

In view of its important application to clinical studies, especially in connection with heparin therapy, many investigators (Reiner, de Beer and Green, 1942; Shelley, Hodgkins and Visscher, 1942; Jaques, 1949; Jorpes, Kallner and Bostrom, 1949) have tested the toxicity of protamines in various animals. In the rat, mouse and guinea pig, the protamines produce anaphylactoid effects with intravenous L. D. 50 doses of about 100 mg./Kg. Shelley et al (1942) explain this effect as being due to embolic vascular phenomena such as agglutination of the blood corpuscles and precipitation of fibrinogen. Jaques (1949) does not believe that these are responsible for the toxic effects in the dog. Jaques' observations led him to believe that the sharp fall in arterial pressure caused by protamine (salmine) was probably due to two main factors: (a) a reflex vasodilation which initiates the fall and (b) a release of pharmacologically

active substances like histamine. Unfortunately, Jaques was unable to substantiate clearly his belief that histamine was released, as increased plasma histamine levels could not be found after the injection of protamine. His best argument for the release of histamine by protamine was that, on occasion, benadryl lessened the severity of the shock.

It is notable that while Jaques based his hypothesis on the fall in arterial pressure caused by protamine, ours was based on the observation that protamine, like other known histamine releasers, potentiated the effect of DA_{10}, which is itself a potent histamine releaser.

As well as the protamines, we were also able to investigate the related synthetic substance, polylysine, whose synthesis, chemical properties and certain physiological actions have been described by Stahmann and co-workers (see Rubini, Becker and Stahmann, 1953, for references).

To test our theory, three types of experiments were employed: (a) The perfusion, by the protamines, of the cat skin preparation as described by Feldberg and Paton (1951)*; (b) The sampling of dog liver cell-free suspensions incubated with the protamines and polylysine, for histamine estimations*; (c) The sampling of blood for plasma histamine determinations following injections of the protamines, salmine and clupein, and polylysine.

* The author wishes to acknowledge the collaboration in these experiments of his colleagues Dr. H. Garcia-Arocha and A. L. Grossberg, who conducted the cat skin perfusion and cell-free liver suspension experiments.

The protamines have the property of interacting strongly with body proteins at a neutral pH, as might be expected from their high isoelectric points. This combining power, well-known for protamines, has also been reported for polylysine by Stahmann et al. Such considerations made it apparent that injecting the compounds into the blood stream of the intact animal would be a rather inefficient way of assuring that they reached the sites of histamine release in the cells outside the vascular wall. Therefore, in addition to experiments on intact dogs and cats, the other two preparations were employed to obviate some of the above-mentioned difficulties.

That a cell-free liver suspension might provide a source of bound histamine was suggested by the observation of Trethewie (1938) that grinding dog's liver with sand did not release all the bound histamine. Such a preparation would, of course, overcome the complications, firstly, of retention of substances in the blood stream and, secondly, of limited diffusion of compounds through the intact cell wall.

The method of preparation, as devised by Mr. Grossberg, consists of grinding liver, which has been perfused blood-free, with sand in ice-cold Tyrode , pH 7.7-8, straining out the sand and larger particles, and centrifuging down the fine cell fragments at 6000 rpm. for 15 minutes. The particles thus obtained are washed twice with cold Tyrode by centrifugation and decantation and the final suspension made up in Tyrode , in a volume giving approximately 2 gm. original weight of liver per ml., and kept at 0° C. until use. The suspension contains from 5 to 20 μg bound histamine per ml. The histamine may be released completely from the particles by boiling. At 37° C.

histamine is released spontaneously at a slow rate, while in the presence of a known histamine releaser such as DA_{10}, in concentrations which are active in the intact animal, histamine is released at a much higher rate. This release is, however, much slower than in the intact animal, complete release with DA_{10} at 0.1 mg./ml. being attained only after about one hour.

To study the action of releasers on this preparation, they are incubated with aliquots of the suspension. At the end of the incubation period, the suspension is centrifuged, the supernatant decanted, boiled, diluted from 10 to 50 times, and assayed for histamine on the guinea pig ileum.

Figure 31 is a graph of the rate of release of histamine from the liver cell fragments by clupein and salmine, compared with the rate of release by DA_{10}. The clupein and salmine were in the form of the neutralized sulfates, and were judged to be histamine free since they gave no contraction when tested on the guinea pig ileum. The concentrations of the substances were chosen, on the basis of previous experiments, to give approximately equal rates of release. It may be concluded that, on a weight basis, clupein is approximately 5 times, and salmine 40 times, less effedtive than DA_{10} in liberating histamine from this preparation. Assuming a molecular weight of 2000 for clupein, the latter is equal to or more effective than DA_{10}, mole for mole, as a histamine releaser. The lower curve, labelled "control", demonstrates the spontaneous rate of histamine release in the preparation.

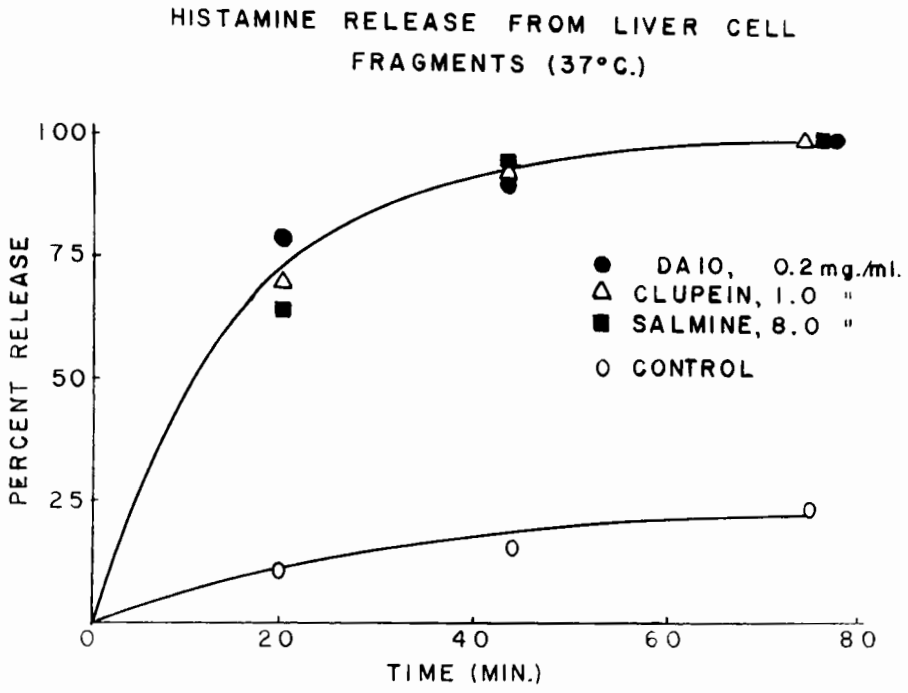


Figure 31

The release of histamine from liver cell fragments by DA_{10}, clupein and salmine. The total amount of histamine, 12 μg., was contained in a suspension of liver cell fragments having a volume of 5 ml. See text for other details. (Experiment and Figure with the permission of A. L. Grossberg)

A similar experiment in which the effect of polylysine was compared with that of DA_{10} demonstrated that the rate of histamine release by 0.4 mg./ml. of polylysine is approximately equivalent to that produced by 0.2 mg./ml. of DA_{10}. The polylysine, generously provided by Dr. Stahmann, consisted of polypeptides with an average chain length of 15 residues, so that mole for mole it was about 5 times more effective than DA_{10} as a histamine liberator.

For the investigation of histamine release in the perfused cat's skin, the preparation was set up as described by Feldberg and Paton (1951). The skin, attached vessels, and the Ringer-Locke perfusion fluid were kept at 38° C. The perfusion pressure was maintained at about 100 mm. Hg, giving a perfusion rate of 1-1.5 ml./min.

Figure 32 is a graph of the histamine output from this preparation as a result of the introduction of 100 mg. of clupein sulfate into the perfusion fluid at a maximum concentration of 20 mg./ml. There was an explosive release of histamine, amounting to 61 µg after 36 minutes. A control period of perfusion for 30 minutes beforehand caused no detectable histamine release. The proof that the active substance appearing in the perfusate was histamine consisted of the fact that the perfusate samples were equally active in comparison to histamine when assayed on the atropinized guinea pig ileum and on the blood pressure of the atropinized cat; the responses in these two assays were reduced proportionately with histamine by neoantergan; and finally, the active substance was resistant to boiling in 2N HCl for 90 minutes. A maximum histamine concentration of 3.0 µg/ml. was obtained in the first 5 minute sample. The reduction in output noted during the subsequent 10 minute period was due to the intense vasoconstriction in the preparation, occurring as a result of the histamine

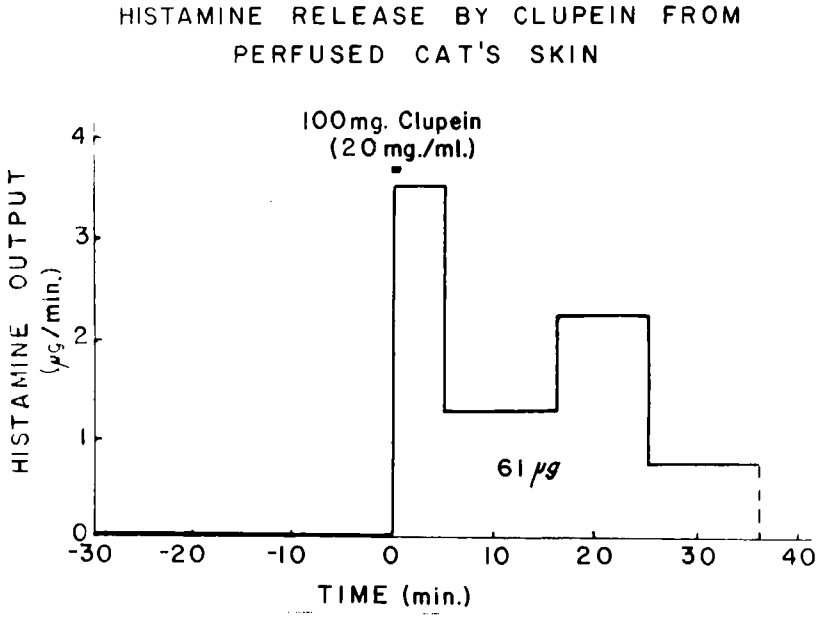


Figure 32

The release of histamine by clupein from the perfused skin. The skin was removed from a cat's leg together with its supplying blood vessels. The preparation was then perfused with warmed Tyrode solution at a pressure of about 100 mm. Hg. and the perfusate collected for the estimation of histamine. See text for other details.

release--an effect noted by Feldberg and Paton (1951) in their experiments, during high rates of histamine release by propamidine and 48/80.

The effect of intravenously administered polylysine and protamines was tested in cats and dogs. These compounds were injected through the femoral vein into animals under chloralose or nembutal anaesthesia and blood samples were taken from the femoral artery at 1, 3 and 30 minutes after the injection. Blood taken before the injection was used as a control sample.

A 10 mg.% solution of polylysine HCl was injected at doses ranging from 3.7 mg./Kg. to 10 mg./Kg. Figure 38 contains data from a representative experiment. The injection of 3.7 mg./Kg. of polylysine into a dog caused a fall in the blood pressure which started about 20-25 seconds after the substance entered the blood stream. The plasma sample obtained before the injection could not be shown to contain any histamine, whereas the sample collected 1 minute after the injection had a histamine equivalent of 0.03 µg/ml.

In studying the effect of the protamines on intact animals, the protamine sulfates were dissolved in saline, neutralized with NaOH, and the solutions filtered. From 30 mg./Kg. to 100 mg./Kg. of protamine at a concentration of 20 to 40 mg./ml. was administered intravenously. The injection time was 10-30 seconds.

In cats and dogs in which the blood pressure was recorded, we noticed that following the injection of protamine, the characteristic fall of arterial pressure took place after a delay of about 25 seconds. When clupein was injected through the portal vein of a dog, the hypotensive

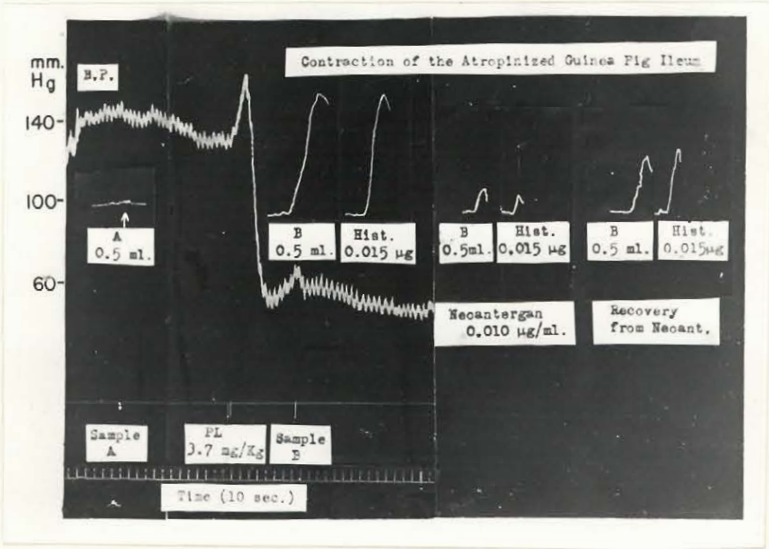


Figure 33

The action of polylysine on the blood pressure of a dog. Inserts show the action of plasma samples, taken before and after the polylysine injection, on the guinea pig gut. The gut contracting substance in plasma sample B is identified as histamine by the Neoantergan test. One ml. of plasma B contains 0.03 µg. histamine.

effect took place after 18 seconds (Figure 34). This more immediate action of the protamine administered through the portal vein, when compared to its action when injected in the femoral vein suggests that the compound is acting to release histamine from the liver, claimed to be the organ in the dog from which occurs the liberation of histamine. Figure 35 illustrates further the similarity of the protamine clupein to the known histamine releaser, Me_2DA_{10}. The time at which the response to protamine occurs is about the same as that for Me_2DA_{10}, while the time taken for an equi-depressor dose of histamine is much shorter. The non-specific depressor effect of the protamine which occurs before the secondary response may be due to a reflex vasodilation of the arterioles as indicated by Jaques (1949). The approximate ratio of potency by intravenous injection of Me_2DA_{10} to clupein is 1:70. Since the strength of Me_2DA_{10} is about 1/3 that of DA_{10}, then DA_{10} is about 200 times stronger than clupein on a weight basis.

In contrast to what we have found with the liver particles and in the cat skin preparation, in the intact animal only a slight increase in plasma histamine was detected after the injection of protamine.

Table 8 summarizes our experience in this respect. Of a total of 18 animals injected with protamines, only 8 were found to have detectable increases in plasma histamine following the injection. These increases, amounting to 0.01 - 0.9 µg/ml. histamine, were found in the samples taken 1 minute after the injection. The histamine content of subsequent samples was always less than that of the 1 minute sample. The plasma histamine was elevated in all 3 animals injected with polylysine.

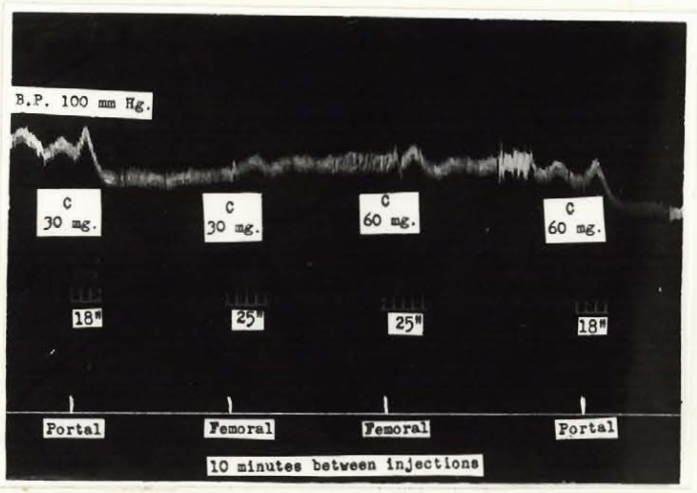


Figure 34

The action of clupein, when given by portal and femoral routes, on the arterial pressure of a dog. The time of the response is much shorter when the portal route is used for the injection. This is because the histamine is released from the liver--not so quickly reached by injections given via the femoral route. The smaller effect of clupein when given via the femoral route is because a lower concentration of clupein passes through the liver in its initial circulation.

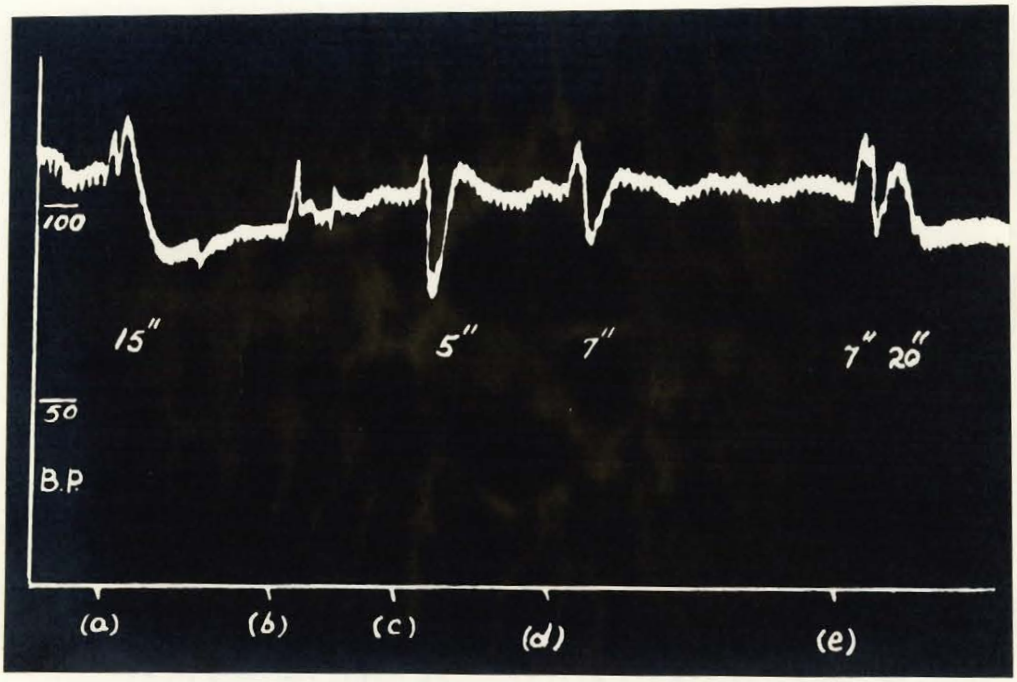


Figure 35

The comparative action of clupein, histamine and Me_2DA_{10} on the blood pressure of a chloralosed cat, 3.2 Kg.

(a) Me_2DA_{10}, 1 mg.; (b) Saline, 2 ml.; (c) Histamine, 1 g.; (d) Clupein sulfate, 30 mg.; (e) Clupein sulfate, 70 mg. Photostat of original record. Please see text for further details.

Table 8

PLASMA HISTAMINE AFTER INTRAVENOUS INJECTION

OF POLYLYSINE AND PROTAMINES

Releaser (No. of Expts.)	Dose (mg./kg.)	Animal	No. of positive results	Plasma Histamine after injection[a] (μg./ml.)
Polylysine (3)	3.7	Dog		0.03
	3.7	Cat	3	0.08
	10	Cat		0.065
Clupein (10)	30	Dog		0.9
	30	Dog		0.05
	30	Cat	4	0.01
	40	Cat		0.03*
Salmine (8)	30	Cat		0.06
	30	Cat		0.02
	50	Cat	4	0.06
	100	Cat		0.012*

* Animal died within 10 minutes after the injection

a/All plasmas before injection contained less than 0.005μg./ml. histamine.

Some of the plasma samples obtained both before and after the injection of protamines contained a substance capable of producing a contraction of the guinea pig ileum, but this action was not abolished by atropine or Neoentergan. The nature of this contracting substance was not investigated.

The weak, and inconsistent, histamine releasing action of protamines when given intravenously, contrasting with the powerful effect of these substances in liberating histamine from the washed cell free homogenate and from the Ringer-Locke perfused skin, could be explained by the fact that, when administered intravenously, a large proportion of the injected protamine binds to the plasma proteins and only a small fraction reaches the sites from which histamine is released.

These observations on the histamine releasing action of basic polypeptides are of interest from several points of view. Firstly, the histamine releasing action of both protamines and polylysine is identical with that of licheniformin, a polypeptide antibiotic, and Witte's peptone. The similarity in the structure of these related compounds suggests the possibility that, perhaps, some naturally occurring polypeptides could play a role in the natural mechanism by which histamine is released from the tissues as in the antigen-antibody reaction.

Secondly, the ability of strongly basic polymers such as protamines and polylysine to combine with the acidic polymer heparin, deserves some attention. This action has long been known in the case of protamines, and has been observed by Stahmann and coworkers and others in the case of polylysine. Equally true, and perhaps less well known in this respect, is the fact that many of the other compounds of

simpler structure which release histamine, also combine strongly with heparin. We have observed that within a rather narrow concentration range, DA_{10}, propamidine and 48/80 all form insoluble complexes in vitro with commercial heparin. Further, outside these concentration ranges, the formation of soluble complexes of heparin with the histamine releasers can be demonstrated. This is done by the use of the dye Azure A which when combined with heparin exhibits metachromasia. If DA_{10} is added to a heparin solution in amounts that cause no precipitation, the addition of Azure A will now not produce as much metachromasia as it would in the absence of DA_{10}.

These facts take an additional meaning in view of the recent work of Riley and West (1953) who have found that tissues rich in mast cells, the site of heparin in the body, are also rich in histamine. The accumulating evidence points to the probability that histamine, too, occurs in the mast cells. The close proximity of bound histamine and of heparin in the body, when considered in the light of the heparin combining power of histamine releasers, tempts one to postulate that the combination of a basic substance with heparin may lead to the release of histamine.

The following hypothesis may be put forward:

(1) It is postulated that there exists a complex between a tissue enzyme and heparin--the combination being enzymatically inactive. In support of this postulate, enzyme-inhibiting action of heparin on the plasma enzyme, fibrinolysin, has been described by Astrup, Crookston, and MacIntyre (1950). Also, Glazko and Ferguson (1940) have shown that heparin inhibits the proteolytic action of trypsin.

(2) The combination of a naturally occurring polypeptide histamine releaser with the heparin attached to the enzyme, removes the

heparin from the enzyme and renders the latter active.

(3) The active enzyme initiates reactions leading to the release of bound histamine.

Although such a hypothesis fits well with many of the known facts concerning histamine release and the inhibitory effect of heparin on this release (Dragstedt, Wells, and Rocha e Silva, 1942), there are, nevertheless, a number of facts which at present seem to speak against it:

(1) Although all the histamine releasers thus far tested show heparin-combining power, not all known heparin-combining substances can be shown to release histamine. One such substance is the dye, toluidine blue. However, this substance has been investigated as a histamine liberator only by intravenous injection in the intact dog, and such a method is open to the objections mentioned previously for protamines, since the dye also combines strongly with plasma proteins.

(2) Commercial heparin, in doses up to 40 mg./Kg. in intact cats, and in concentrations up to 20 mg/ml. in the <u>in vitro</u> liver cell fragment preparation, does not inhibit histamine release. However, a slight inhibition occurs at heparin concentration of 40 mg./ml. in the <u>in vitro</u> preparation. MacIntosh andPaton (1947) reported that doses of 40-60 mg./kg. reduced the hypotensive effect of licheniformin in intact cats.

It should be stressed that the action of intravenously injected heparin is again complicated by the fact that it, too, strongly binds to plasma proteins. In addition, commercial heparin is lacking in some factors present in native heparin, which Snellman and coworkers (1951) have shown to occur as a lipoprotein complex. They have shown, further

that in the absence of its lipoprotein complement, heparin does not produce the antithrombic effect. The lact of required cofactors may also explain the inability of commercial heparin to inhibit histamine release in our in vitro studies.

In view of the present hypothesis, the natural occurrence, in the animal's body of such basic compounds as the simple polypeptides examined, might allow them to be implicated in several reactions in which histamine is released such as anaphylaxis and allergic phenomena. Also, the role of heparin as the natural inhibitor of the fibrinolytic mechanism in the body can now be considered. However, the participation of heparin in instances of histamine release in other animals has not been demonstrated.

The Release of Heparin by Protamine.

At the time of their histamine-releasing action, histamine liberators also cause the release of heparin. Since it has been shown that the protamines are histamine-releasing agents, it can be supposed that they also cause the release of heparin. However the intense combining action of heparin with protamine (Jaques, 1943) seems almost prohibitive of estimating the release of heparin with either clotting or other methods since the plasma would contain varying amounts of protamine either free or bound with plasma proteins and heparin. Protamine itself has an anticoagulant effect on the blood by virtue of its combining action with fibrinogen and thromboplastin. (Chargaff, 1938).

A few preliminary experiments served to show that the raised clotting times caused by large injections of protamine could be either decreased or increased by the in vitro addition of heparin

or protamine respectively. It was also shown that while protamine possessed no metachromatic activity itself, it was able to lower that caused by heparin. With these two bits of information then, the following experiment was performed to determine whether the intravenous injection of protamine, into a dog, caused the release of heparin.

Initially 25 ml. of fresh dog's blood was added to 1/10 volume of 3.8% sodium citrate and centrifuged to provide standard plasma for metachromatic determinations. 25 ml. samples were again taken at 1, 3, and 10 minutes after the injection of 30 mg./Kg. of clupein into the 13 Kg. dog. 10 ml. of each sample was mixed with citrate and centrifuged immediately to obtain the plasma for metachromatic comparisons. Clotting times were made with the remaining blood by itself and also after varying amounts of heparin or protamine had been added to it. The following results were obtained.

Table 9

Dog Blood Clotting Times after the Intravenous Injection of Protamine (Clupein)

Small tubes already contain saline, heparin or protamine (0.2 ml.) The withdrawn blood (samples 1, 3, 10.) has been calculated to contain about 0.3 mg. of the injected protamine. Values in minutes.

Time of Sample		Added Heparin (mg.)					Added protamine (mg.)				
	n	.005	.02	.04	.07	.10	.02	.05	.10	.30	.50
0	10	65	>90	>90	>90	>90	14	15	20	20	31
1	35	31	25	12	14	55	45	47	50	50	70
3	31	24	17	15	>90	>90	36	56	60	>90	>90
10	16	>90	>90	>90	>90	>90	29	27	38	45	75

Table 10

Metachromatic Activity of dog Plasma After the injection of Protamine (Clupein)

Time of Sampling (Minutes)	0	1	3	10
Plasma Density readings after the addition of the following to 1ml. of Plasma.:				
Saline (0,2 ml.) (n)	.380	.430	.411	.473
Heparin (0.02 mg.)	.638	.600	.542	.670
Protamine (0.5 mg.)	.385	.382	.392	.418
+ Heparin (0.02 mg.) and Protamine (0.5mg.)	.405	-	-	-

The clotting time results for the normal (n) sample indicate that one of two mechanisms is causing an increased clotting time.

(1) a release of heparin has occurred.

(2) the protamine itself is causing an increased clotting time.

It is soon apparent that the increased clotting times are due to the action of protamine because the addition of varying amounts of heparin to the tubes decreases the clotting time, and protamine added to similar samples caused only a further increase in the clotting time.

It is readily seen that the increased clotting time of the 3 and 10 minute samples are also due to protamine. But why should their clotting times be less than that of the 1 minute sample? Is it because there is more heparin in the blood at this time - or less protamine?

Table 10 shows that the increased metachromasia in the 3 and 10 minute samples are all further increased by the addition of heparin or reduced by the addition of protamine. Since protamine itself has no influence on the reading of the plasma sample that contains no heparin it must be concluded that the increased metachromasia of the other

samples is due to the presence in them of heparin.

It appears that the 10 minute sample has more metachromasia than the 1 minute sample, but unfortunately, the figures for the three minute sample do not add to this observation. In any case the higher metachromasia in the 10 minute sample can not be used to determine whether more heparin, or less protamine, is in the plasma. Other methods to elucidate this matter were not attempted.

In this experiment the 1 minute sample was also assayed for histamine by means of the cat preparation. 0.6 µg./ml. was found, but unfortunately, there was not sufficient plasma to check this result against neoantergan.

Conclusion: The injection of a large amount of clupein into a dog increased the clotting time to several times the normal value. This increased clotting time was found to be due to the injected clupein. The presence of heparin in such plasma was indicated by the increase in them of metachromatic activity.

PART 3. DISCUSSION

During the presentation of the experimental results the implication of the findings reported were discussed. Therefore only a general consideration of the more important aspects of the work need be given here.

At the beginning of the study it was proposed that the author search for pertinent facts which might be used in the understanding of the liberation of histamine. "Histamine releasing agents" were to be used as the means of approach. It is now clear that this group of substances are valuable tools for studying problems involving the release of histamine.

The author first determined if the previously found actions of these substances were reproducible in his hands. In doing so a new property of histamine releasing substances was found, i.e., that one histamine liberator could potentiate the effects of another even if the former was injected several minutes before the latter.

The finding of this property proved of great value in that it led to the discovering of the histamine releasing action of the protamines, which discovery, in turn, allowed the formulation of a workable hypothesis for a part of the reaction which brings about the release of histamine.

Briefly, the hypothesis is as follows: The combination of "histamine releasing substances" with heparin, proposed to be attached to a cellular enzyme, removes heparin from the enzyme and renders the latter active. The active enzyme then initiates the mechanism which finally releases histamine.

In the introduction, a general survey was made of various substances and events by which the release of histamine is initiated.

The similarity in a number of animal species of the events leading to histamine release had led the author to believe that the liberation occurs by activation of the same mechanism; namely, an enzyme system capable of freeing histamine from the cell.

Unfortunately the proposed theory has one drawback that prohibits its immediate application. This is, that in no species has heparin been shown to have a causative relation to the events which lead to a release of histamine. However, in the dog, heparin and histamine are released together in anaphylactoid reactions and this suggests that heparin may be involved in the reaction. The correlation of the amounts of heparin and histamine in rat tissues with the mast cell count in those same tissues as shown by Riley and West (1952) and Riley (1953) might be taken as suggestive evidence that heparin and histamine have related cellular function.

A few authors have observed that raised clotting times occur in the cat and guinea pig during anaphylaxis but the reason for this has never been explained. Since methods for the estimation of heparin are not difficult to perform, such experiments would soon determine if heparin has any part, actual or coincidental, in histamine releasing actions of other species. The participation in this reaction of other body polysaccharides related to heparin, but not necessarily having any anti-clotting action, should also be determined. It is notable that MacIntosh and Paton (1949) did not observe an increased clotting time in the blood of cats after the cats had been injected with histamine liberators.

On the two observations made which speak against the proposed theory for histamine release, the apparent failure of Toluidine Blue to release histamine in the intact animal cannot be used as a strong

objection. Other techniques, which are described in the text and which seem more suited for testing drugs for the action of histamine release, should be used to determine if Toluidine Blue does possess histamine releasing activity. The value of such methods as the skin perfusion technique and the liver cell fragment test for estimating histamine releasing potency is clearly greater than that of the intact animal preparation - especially if the histamine-releasing activity is low, as in the case of the protamines. One observation that makes the investigation appear more hopeful is that Campble (1952) has shown that thrice weekly injections of Toluidine Blue were able to cause the disruption of mast cells, - which cells are proposed by Riley and West (1952) to contain histamine in large amounts. Campble's observation agrees with the work of Riley (1953) who used the fluorescent histamine liberator stilbamidine to show that histamine and heparin probably have a common cellular origin.

The second observation which appears to cast doubt on the proposed theory is that large amounts of heparin do not inhibit the release of histamine in intact animals nor in the liver dust preparation. In the experimental part the possibility was raised that the absence of essential cofactors of heparin, in commercial preparations of heparin, may be the cause of heparin having no inhibitory effect on the reaction. The importance of determining whether a cofactor does actually permit this supposed action of heparin should not be overlooked. The investigation of the inhibitory action of various sulphuric acid esters possessing heparin like properties is also desirable.

"Skin Reactions" in the experimental section of the thesis show that "histamine liberators" may have quite different actions when tested on species other than that used when the initial characterization was made. The skin reaction tests showed that 48/80 had no local action on guinea pigs, but that DA_{10} and its homologues had a very harmful local effect. On the other hand 48/80's lethal effect by weight was much greater than that of DA_{10}. It could be postulated that DA_{10} caused the activation of a proteolytic enzyme to produce the local skin lesion but no such activation appeared to take place with sublethal doses of 48/80 or, in an isolated test, with such histamine releasers as protamine, atropine and neoantergan. A worth while study would be to characterize histamine liberators as to production of their effects in different animals.

The significance of having found that simple peptides such as the protamines and polylysine release histamine will probably not be known for some time. However, it may be easier to attack some allergic problems knowing that such polypeptide substances in the body may be responsible, in some way, for initiating allergic reactions.

Of the compounds tested as histamine release inhibitors, perhaps suramin has the simplest and most easily explained mode of action. As Guinaraes and Lourie (1951) had shown while investigating the hypotensive action of pentamidine, the inhibitory action of suramin on pentamidine's histamine liberating activity is probably due to the formation of a precipitate, which action, however, was not believed to prevent the desired pharmacological effects of pentamidine.

Inhibitory action against histamine liberators is very desirable since a large number of useful drugs which cause the release of histamine cannot always be injected without the occurrence of this unpleasant side action. However the intravenous injection of such precipitates

as that formed by suramin when it is mixed with pentamidine (or DA_{10}) is quite contrary to medical practice. Thus, if histamine liberators were ever to be used as therapeutic agents, the intravenous injection of them with inhibitors as complexes (in order to prevent an immediate release of histamine in large quantities) would only be feasible if the complex was soluble.

The desired inhibition of the action of histamine releasers, without precipitation, may be found with such polybasic acids as adipic and its homologues. Adipic acid appears to have a liberator-inhibiting effect of short duration but without the formation of a precipitate. It was not determined if a soluble DA_{10} - adipic acid complex was formed but this seems to be a likely possibility. However, it was also noticed that the inhibiting effect of adipic acid also prevented the usual action of histamine. In lieu of a more natural type of histamine release inhibitor such as heparin, adipic acid-like substances should be investigated for their use as adjuvants in therapy where histamine-liberation is not desired.

The clinical use of Splenin A in allergies and similar conditions seems quite feasible. Splenin A appears to act reasonably quickly as well as having a powerful inhibitory action on histamine release, and given intramuscularly, its action lasts a moderately long time. It would be desirable to test whether the compound could also be given orally by capsules.

Cortisone, which appears to have the same type of action as Splenin A and is believed to act by the same mechanism has already come into use for the treatment of various allergies with some success. However, cortisone has a wide variety of actions in the body and its effect against the mechanism of allergy is probably not very specific.

Thus far to my knowledge, Splenin A has only been given limited experimental trials with laboratory animals and therefore its range or specificity of action cannot be known. As well as having an active part in the hormonal control of the fibrinolytic system, Splenin A may well have other important physiological functions.

It would be interesting to determine if the moderate inhibitory effect of cortisone against histamine release in dogs would be paralleled by a similar modification of the heparin releasing action of a histamine liberator.

Bloom, (1952), Cavallero and Braccini (1951), and Asboe-Hansen (1952) all claim that cortisone decreases tissue mast cell numbers and therefore the tissue stores of heparin also become smaller. When an animal is naturally refractory, or is caused to be so by various means to the action of histamine liberators, it may be tempting sometimes to attribute this either to tissue histamine depletion or to a lack of the necessary histamine releasing protease. Therefore it seems worth while to determine if tissue levels of histamine and protease are paralleled by that of heparin, and also whether the refractoriness can be correlated with a decrease in any particular one of these substances.

The search by Dr. I. Levi of Fresst and Co. to find low molecular weight protease inhibitors possessing steric resemblance to natural

tryptic substrates is hoped to be continued. Such compounds as the phenylpropane derivatives seemed to have many of the earmarks of such an inhibitor but unfortunately none of the compounds tested were found to possess more than token activity. Phenyl propionate itself should have been tested during this investigation. If such compounds did not possess general toxicity they might constitute a new and useful weapon for the treatment of diseases containing important components of allergy or hypersensitivity.

It is very probable that the histamine releasing activity of some otherwise very useful drugs might prevent them from being given a clinical trial. The addition of methyl groups to the amine moieties of such histamine liberating compounds as those of the alkyl diamine series appears to lower histamine liberating activity several times. Therefore this modification in structure might be used to decrease the undesirable histamine releasing side effect of many valuable drugs to allow their more useful pharmacological actions to be manifested without interference. However, it is possible that such a modification might also change these useful properties. Further increases of the length of the amine side chain of these compounds should also be tested for their effectiveness in decreasing the histamine releasing side action.

The experiments to show if histamine liberators act most strongly in the liver may have been of more practical value to the author than to the problem in general, in that they have provided him with much useful experience in various operative techniques. The author has shown that, in the intact dog, histamine releasers usually act most strongly in the liver but they also can release histamine from other body sources. No correlation was found between the amount of liver histamine and the amount of histamine released into the blood stream after injection of the

histamine liberator. In some experiments this may have been due to a release from sources other than the liver but, in general, no explanation is possible, for the results obtained.

In these experiments a glass shunt, devised to exclude the liver from the circulation, and siliconed to prevent clotting, proved very useful. The technique involved is much simpler than the classical methods outlined by Markowitz (1937) and can be used with little instruction by the inexperienced operator provided the usual precautions are observed. This technique is recommended for use in other varieties of investigations on the liver.

Finally, the author would like again to emphasize the value of histamine releasing drugs in investigations where the reproducible release of limited amounts of histamine is desired. In contrast to the uncontrollable anaphylactic reaction a threshold effect can be obtained with histamine releasers and be repeated for several hours. The use of this technique for characterizing antihistamine substances, antihistamine-releasing substances or histamine release potentiating substances is recommended in further studies of this nature.

SUMMARY

1. A brief history of histamine research has been outlined and the the release of histamine in metabolic activity, anaphylactic shock, anaphylactoid shock and trauma has been reviewed. Evidence for the participation of enzyme factors in all of these events has been presented.

2. The so called "histamine liberators" which are believed to release histamine without tissue injury have been reviewed in detail. The many similarities between anaphylactic reactions and the manifest actions of "histamine liberators" suggest that these two conditions produce their effects in the same way: ie. by the activation of an enzyme system. The term "anaphylacto-mimetic" agent has been suggested as a better designation for compounds having the typical "histamine releasing" action.

3. Special emphasis is laid on the observation that whereas anaphylactic like conditions are usually uncontrollable, the amount of "histamine liberator" administered can usually be regulated to produce any desired degree of effect.

4. In the experimental work procedures for the characterization of any substance as a "histamine liberator" have been outlined: and the characteristic effect obtained is described with the aid of records obtained in typical experiments.

5. One new effect that may be used to characterize "histamine liberators" had been described in detail. This is briefly as follows: when subthreshold doses of two "liberators" are injected into an animal at the same time or several minutes apart, their effects summate and the characteristic fall in arterial pressure is observed. The ability of the "liberator" to summate is apparently

independent of its structure or other pharmacological actions.

6. The action of the antihistamine, neoantergan, against the "histamine releasers" is probably an unspecific one: the antihistamine exerts its effect against the histamine released rather than against the histamine releasing reaction of the "releaser" used. Since neoantergan itself is probably a histamine releasing agent as was determined by the above mentioned characterization test, (5), it is doubtful that it could inhibit the reaction in which it, itself plays an active part.

7. As with neoantergan, so also atropine, known already to be a "histamine liberator" (Schachter, 1952) caused potentiation of the hypotensive effect of DA_{10}.

8. The subcutaneous and intradermal injections of "histamine liberators" into guinea pigs has shown that histamine liberators may have very different local effects. Very harsh effects of one "liberator" may not be observed even with lethal doses of another "liberator".

9. A simple method for estimating the release of skin histamine in µg./sq. cm./min. by various "histamine liberators" or antigens has been described.

10. The main site of histamine release in dogs that receive intravenous injections of histamine liberators is the liver but histamine release may also occur in other tissues. A siliconed glass portal shunt especially designed to exclude the liver from the circulation has proven very useful in these experiments.

11. A small proportion of dogs used in "histamine release" experiments did not show the usual hypotensive response to injections of a "histamine liberator". A search for naturally refractory dogs using a variety of techniques was fruitless.

12. In an attempt to cause refractoriness to "histamine liberators" in cats, a number of substances given by intravenous injection were found to be of little value. Some of these substances were: Typhoid-Paratyphoid (AB) Vaccine, Acetyl Salicylic Acid, Ethyl Alcohol, derivatives of phenylpropane, sodium citrate, and Soya Bean Trypsin Inhibitor.

13. Suramin was found to have an inhibitory action of short duration on the histamine releasing action of DA_{10} in the cat. This appeared to be due to the formation of an insoluble Suramin - DA_{10} complex. Homologues of adipic acid appeared to have an inhibitory action similar to that caused by Suramin. Although no precipitate was formed by mixtures of adipic acid and DA_{10}, one cannot exclude the possible formation of a soluble complex.

14. Cortisone given to dogs for 2 days in divided doses (24 mg./Kg./day) was found to have an inhibitory effect on the action of "histamine liberators" but only if the animal had been moderately depleted of "readily available histamine" by an earlier dose of "histamine liberator". The observation supported the belief that cortisone participates in the hormonal regulation of the fibrinolytic system believed to initiate the release of histamine.
Histamine levels in the skin of cats that had received a similar course of cortisone treatment were not consistent.

15. Splenin A was found to exert a reasonably potent inhibiting effect on the action of histamine releasing agents. Effects were obtained with both water soluble and oil soluble preparations of Splenin A. The observations substantiate the belief that Splenin A, in addition to cortisone, plays a role in the system regulating fibrinolytic mechanisms.

16. Some pharmacological properties of a group of bis-mono-methylated and bis-di-methylated aliphatic amines have been examined. Their ability to release histamine in the cat, and histamine and heparin in the dog, has been demonstrated. Qualitatively, they appear to have the same general action as their non-methylated analogues but no conclusion could be reached for their quantitative effects. Toxicity tests suggest that the lethal action of these compounds in rodents is not due to the release of histamine.

17. The low molecular weight proteins; clupein, salmine and poly-lysine have been shown to possess histamine liberating power. The concomitant release of heparin with histamine in dogs which had received large doses of clupein was suggested by metachromatic tests. However, the increase in clotting time following the injection was found to be due to the clupein itself.

18. A theory to explain part of the mechanism whereby histamine is released has been proposed. It is postulated that the combination of "histamine liberators" with heparin assumed to be attached to a cellular protease removes heparin from the enzyme and renders the latter active. The active enzyme then initiates the release of histamine.

Note: The claims to original research have been included in the above summary.

APPENDIX

Chemical compounds which have been shown to cause the release of histamine. Allergens, venoms, and toxins are not included.

Adrenaline	Eichler and Barfuss (1940)
	Staub (1946)
Adrenochrome	Kuschinsky and Hille (1952)
Antrycide	Feldberg and Paton (1951)
Atropine	Burstein and Parrot (1949)
Benadryl	Arunlakshana (1953)
Benzamidine derivatives	MacIntosh and Paton (1949)
Bile salts	Schachter (1952)
Cinchophen	Slutzky, Dietz, Stoner, and Loughlin, (1952)
Chloroform	Lambert and Rosenthal (1943)
"Compound 15"	Collier and MaCauley (1952)
Curare	Alam, et al (1939)
Diamines, diamidines, diguanidines, etc.	MacIntosh and Paton (1949)
Egg Albumin	Feldberg and Schachter (1952)
48/80	Paton (1949)
Horse serum	Feldberg and Schachter (1952)
Haematoporphyrin + light	Feldberg and Talesnik (1953)

Licheniformin	MacIntosh and Paton (1949)
Leukotaxine	Dekanski (1949) Miles and Miles (1952)
Laudolissin	Collier and McCauley (1952)
Morphine and opium alkaloids	Nasmyth and Stewart (1949) Feldberg and Paton (1949)
Mono-alkyl amines	Mongar and Schild (1953)
Methylated alkyl diamines	Garcia and Ashwin (1952)
Mercuric Chloride	Feldberg and Kellaway (1938)
Peptone	Feldberg and O'Connor (1937) Code (1939)
Pentamidine	MacIntosh and Paton (1949)
Pethidine	Schachter (1952)
Priscol	Schachter (1952)
* Protamines (clupein, salmine)	Ashwin, Garcia and Grossberg (1953)
* Polylysine	Ashwin, Garcia and Grossberg (1953)
Quinine	Ambache and Barsoum (1939)
Strychnine	Schild and Gregory (1947)
Stilbamidine	MacIntosh and Paton (1949) Riley and West (1952)

*Shown in present thesis

Tubocurarine	Reid (1950)
Tween 20	Krantz, et al (1948) (1949)
	Goth, Allman, Merrit and Holman(1951)
Turpentine	Rocha e Silva (1940)
2339R. P. and 2786R. P. (anti histamines)	Pellerat and Murat (1946)
Xylene	Lambert and Rosenthal (1943)

Other histamine-releasing substances closely related to those mentioned above are found in the references listed.

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