THE METABOLISM OF HISTAMINE.

by

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THESIS

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GENERAL INTRODUCTION.

Up to the end of the last century, the only mode of functional co-ordination in the organism which was understood was that of reflex nervous mechanism. With the discovery however of specific hormones, the concept that there might be a humoral co-ordinating mechanism began to be entertained. It was Starling who originally introduced the term hormone and defined it as "any substance normally produced in the cells of some part of the body, and carried by the blood-stream to different parts, which it affects for the good of the organism as a whole". (Starling 1914). In the years following, a certain amount of confusion arose in that the term hormone was used to cover a host of pharmacodynamically active substances, many of which were quite hypothetical in nature, and obtained from diverse organs by various modes of extraction. Thus Le Heux (1920) speaks of 'Hormon der Darmbewegung' and Freund (1921) of 'Zellzerfallshormonen' etc. A clear differentiation between these hormone-like substances and specific hormones was made by Trendelenburg (1929) and Dale (1929). It is now generally accepted that the specific hormones are produced in especially developed glandular organs, the glands of internal secretion, and are transported by the blood stream to distant organs where they exert their effect. In contra-distinction to these, there are substances not only produced in any one organ but which are formed in many tissues as well as tissue juices, which do not require any specific type of cell. The name of 'Gewebsstoffe' or 'Gewebshormone' has been given

to these substances by Feldberg and Schilf (1930). They are also referred to as 'körpereigene Wirkstoffe' by Rigler (1934). These 'tissue-hormones' are found under physiological conditions in the body, and they are supposed to act at the site of production or liberation, thus differing markedly from the specific or hormones of internal secretion. It will be seen later that under pathological conditions such tissue hormones may behave in the same manner as specific hormones, in that they may be carried by the blood to act on distant organs. Products of metabolism such as carbon dioxide or lactic acid which may also have a co-ordinating effect on organs distant from the site of production are not considered to come under the category of tissue hormones, according to Dale (1929). Of all the tissue hormones only three are of known chemical constitution. These are histamine, acetyl-choline and aderylic acid and its derivatives. Both acetyl-choline and histamine were prepared synthetically long before their physiological significance was appreciated. Bayer (1867) prepared acetyl-choline, and only in 1906 did Hunt and Taveau show its pharmacological activity. The great discovery by Loewi (1922) that it was liberated by nervous activity and was a chemical transmitter formed the starting point for much of the subsequent work.

Histamine was synthetically prepared in 1907 by Windaus and Vogt and was isolated from ergot in 1910 by Barger and Dale. It was following this that an intensive study of its actions was commenced. In 1929, a group of adenylic acid derivatives was described by Drury and Szent-Györgi, which could be isolated from animal tissue, muscle in particular. Along with these definitely known substances, there exist other tissue hormones of unknown chemical constitution, and of these only two will be mentioned, namely kallikrein (Kraut, Frey and Werle 1930) and the substance 'P' of

Euler and Gaddum (1931). Histamine and acetyl-choline are the most important and are highly active pharmacodynamically.

All of these have common characteristics, namely that they have a vaso-dilator action and are found in various tissues. For this reason, they have been described together in the monograph by Gaddum and Dale (1936). Since many of the actions of this group of substances are very similar it is self evident that considerable care and discrimination must be exercised before one can ascribe any particular action as being due specifically to one of them.

With the increasing interest in histamine, some excellent monographs dealing with various phases of its properties have been written. (Feldberg and Schilf 1930; Best and McHenry 1931 ; Gaddum and Dale (1936).

This thesis is concerned mainly with the metabolism of histamine, and in particular with certain aspects of the mechanism of its detoxification in the animal organism. There now follows a general review of the subject, the detailed headings of which will be found in the index.

HISTORICAL SUMMARY.

"History of the Investigations Leading to the Discovery of the Presence of Histamine in Animal Tissues."

The presence of depressor substances in watery and alcoholic extracts of tissues was noted as early as 1895 by Oliver and Schafer and by Vincent and Sheen (1903) etc. The nature of these substances was unknown, and the existence of a hypothetical substance was postulated by Popielski (1909) which he called 'vasodilatin'. The synthesis of histamine by Windaus and Vogt (1907) and its isolation from ergot by Barger and Dale (1910) was followed by pharmacological studies by Dale and Laidlaw (1910) and the opinion that the actions of these depressor substances might be due to histamine at least in part began to be expressed.

With the growing mass of evidence in favour of histamine being the active principle in tissue extracts, there was still the objection that these experiments were based on biological assay of crude extracts, and the existence in them of histamine as such was not yet proven. Even though Barger and Dale (1911) had isolated histamine from intestinal mucosa in small amounts, it was argued that this might be due to contamination by intestinal contents.

While this controversy was still going on new and interesting theories were being evoked about the actions of histamine in vivo, and their similarity to the conditions of anaphylactic shock (Dale 1913, Coca 1914, Abel and Kubota 1919). Also the work on traumatic shock was considered to

indicate that histamine or histamine-like substances were liberated in this condition from the traumatized tissue and caused the resultant shock-like symptoms (Cannon and Bayliss 1919; Dale 1919).

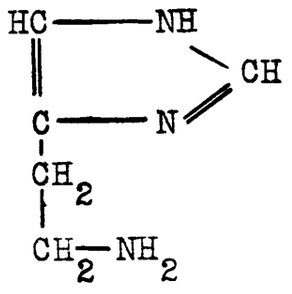
In 1919 Abel and Kubota isolated histamine from dried pituitary power and made the claim that histamine was the active substance responsible for the uterus stimulating property of this extract. However using a different method, Hanke and Koessler (1920) were unable to detect histamine in fresh pituitary glands. After much controversy, it was finally agreed that although histamine may be present in small amounts in the pituitary, it is not the specific factor concerned in the effect on the uterus.

Finally, the controversy as to whether histamine is present as such in animals tissues was settled by the isolation of the picrate salt from ox liver and lung by Best, Dale, Dudley and Thorpe (1927). Later, Dale and Dudley (1929) isolated it as the picrate from ox and horse spleen and Thorpe (1930) from ox muscle.

"The Chemical and Physical Properties of Histamine."

Histamine or beta-iminazolylethylamine was given its name because of the fact that the pharmacodynamically active amine could be formed by the decarboxylation of the amino acid histidine (Windaus and Vogt 1907). It is basic by virtue of the NH_2 and as such combines with acids to form stable salts.

Histamine has the following formula:



The molecular weight of the base is 111. It crystallises in colourless crystals and has a melting point of 83-84°C. It is very soluble in water and alcohol, hot chloroform, but is insoluble in ether. The two most commonly used salts are the dihydrochloride with a molecular weight of 183 (60% histamine) and the diphosphate, molecular weight 307 (30% histamine). The salts differ from the base in that they are not soluble in hot chloroform. Both the picrate and dipicrate salts of histamine have been utilized in the extraction of histamine from tissues since they are not soluble in cold water.

Pure solutions of histamine, or histamine in tissue extracts are stable in concentrated acid solution and may be boiled for as long as one to two hours without any destruction of the amine. However, whereas pure solutions of histamine may be boiled in alkaline solution without losing their activity, tissue extracts soon become inactive following this procedure.

Solutions of histamine are stable for long periods of time providing they are kept in sterile containers in the ice-box or even at room temperature. Once contamination has occurred, potency is rapidly lost.

If a solution of histamine is made in Ringer or normal saline, the pH of the solution will be lowered. This must be taken into account in pharmacological studies.

Although histamine may be formed from histidine by boiling the latter with hydrochloric acid, this does not occur during the comparatively short period of acid hydrolysis used in present methods (Gavin, McHenry and Wilson, 1933)

"The Natural Occurrence of Histamine in Plants and Animals."

Histamine is widely distributed in both plant and animal tissues, but the number of cases in which actual chemical isolation has been done is relatively small. Most of the work has been accomplished by biological methods of assay. A lengthy table of the plant and animal sources of histamine will be found in the monograph by Best and McHenry (1931).

(1) Plant Sources: Yoshimura (1909) was the first to isolate histamine from a plant source. He used soy beans which had undergone putrefaction. Barger and Dale (1910) made the classical isolation of histamine from ergot and this work was confirmed by Kutscher in the same year.

In recent work Holtz and Janisch (1937) have shown that various vegetables, i. e. spinach, tomatoes and sauerkraut contain small amounts of histamine.

(2) Animal Sources: As previously stated, substances of a depressor nature had been reported in animal tissues by many of the early workers (Osborne and Vincent 1900) (Vincent and Sheen 1903). But it was not until the convincing work of Best, Dale, Dudley and Thorpe (1927) that the presence in tissues of histamine as such was established . Further work on the histamine content of various organs has established that this substance may be present in most tissues of the mammalian organism and that the amounts present vary considerably not only in different species, but also in the same organs of different individuals of the same species. In particular is this true of the histamine content of the lung (Feldberg and Kellaway 1937b).

Great difficulty was experienced by earlier workers with the problem of the detection of histamine in the blood. Hanke and Koessler (1924) and MacGregor and Peat (1933) were unable to demonstrate it chemically or pharmacologically. Harris (1927), using a crude method of extraction which failed to account for other vaso-dilator substances stated that the blood contained 0.5 mg histamine per 1 kg.

The presence of histamine in the blood was established by the work of Barsoum and Gaddum (1935a), using biological methods, and a method of extraction which excludes other vaso-dilator substances. In the same year Tarras-Wahlberg (1935a) demonstrated biologically the presence of histamine in the plasma of the blood of the rabbit. The work of Barsoum and Gaddum has been confirmed by other workers. (Ungar, Parrot and Pocoule 1937; Riesser 1937). Code (1937b) also confirmed their work and suggested an improved method.

In their original work on blood Barsoum and Gaddum (1935b) stated that the histamine-like activity of the red blood cells was six times greater than that of the plasma. Anrep and Barsoum (1935b) found a ratio of 10:1 for rabbits blood, and 1:1 for dogs blood for the corpuscle plasma distribution. Barsoum and Smirk (1936) also indicated that the histamine content of the cells was higher than that of the plasma. By means of differential centrifugation, Code (1937c) was able to separate blood into three layers, the plasma above, the packed red blood cells below, and the middle 'white cell layer'. In centrifuged unclotted blood the source of 70-90% of the histamine-like activity was found to be in the white cell layer to which under normal conditions it is apparently fixed. If the blood is allowed to clot, it is liberated into the serum quantitatively and is therefore a part of that group of substances

which are freed from the cellular constituents of blood on clot formation (the 'Frühgift' of Freund, 1920).

In earlier experiments, Freund (1920) showed that different types of substances were released from blood if it were allowed to clot. He divided these substances into two main groups, (1) 'Frühgift'; substances released immediately depending as he thought upon destruction of the blood platelets, and the main action of which is vaso-dilator. (2) 'Spätgift' - substances which gradually appear within the course of a few minutes and have a vaso-constrictor action.

The clotting of the blood does not form this histamine-like substance since it does not alter the total blood histamine. Similarly the precipitation of the proteins of whole blood by the action of trichloracetic acid liberates all the histamine present in the blood but does not cause the formation of any new histamine. In further studies Code (1937c) was able to demonstrate that this activity was limited to the granular series of cells, in particular the eosinophiles, and not to the blood platelets as was previously supposed. Using electrodialysis however Minard (1937a) found that most of the histamine activity of rabbits blood was to be found in the platelets.

The non-granular series of cells (lymphocytes) do not contain any histamine (Code 1937d). Finally Code and Ing (1937) isolated histamine chemically from the white blood cell layer of rabbit blood.

The following table is from Gaddum and Dale (1936).

TABLE I.

Histamine Concentration in Various Tissues
in Milligrams per Kilogram.

ORGAN	SPECIES					
	Man	Horse	Ox	Dog	Cat	Rabbit
Blood	0.04	-	-	0.05	0.01	10
Skeletal muscle	-	1.4; 1,1	4	7	-	-
Smooth muscle	-	7.5	-	35	-	-
Bladder	-	7.8	-	-	-	-
Heart	-	-	(9.4; 18 (9.6; 8.1	4	-	-
Lung	-	35	44; 75	16.5	-	-
Liver	-	2.5; 6.6	5.4; 5	33	-	-
Pancreas	-	1.6; 3	-	-	-	-
Spleen	-	7.5	-	-	-	-
Kidney	-	2.6; 3.3	-	4	-	-
Testicle	-	-	1.8	-	-	-
Ovary	-	-	9	-	-	-
Parotid gland	-	5.3; 6.7	-	-	-	-
Submaxillary gland	-	0.6; 0.5	-	-	-	-
Thyroid	-	0.5	-	-	-	-
Skin (Epidermis (Dermis	24 4	-	-	-	-	-

"The Mechanism of the Formation of Histamine."

A. Chemical:

Histamine was synthesized from histidine before it had been isolated from either animal or plant sources in 1907 by Windaus and Vøgt. The procedure was a complicated decarboxylation of histidine.

Further work on the chemical synthesis of histamine was done by Ewins and Pyman (1911) and Pyman (1911). The first paper dealt with the acid hydrolysis of histidine in which a 20% yield after heating at a temperature of 270°C for 24 hours was obtained. This is of significance in that present methods for the extraction of histamine from tissues are based on the fact that histamine is stable at a low pH when boiled, but since most methods require a short period of hydrolysis, one to one and a half hours, the yield from histidine if any must be very small (Gavin, McHenry and Wilson 1933). Pyman (1911) described the synthesis from diamine acetone with a yield of 29% of the theoretical. By improving Pyman's method, Koessler and Hanke (1918) were able to obtain a yield of 50 to 56% of the theoretical.

The action of ultraviolet light on a solution of histidine was first investigated by Ellinger (1928) who reported a formation of histamine following this procedure. Holtz (1934) further noted that the yield was greater when the action was allowed to take place in alkaline medium than when in acid medium. He was able to isolate histamine as the gold and platinum salt. The yield however was never greater than 1%. It may be that the action of the ultraviolet rays could account in part for this low yield in that Holtz also noted that histamine could be destroyed by them.

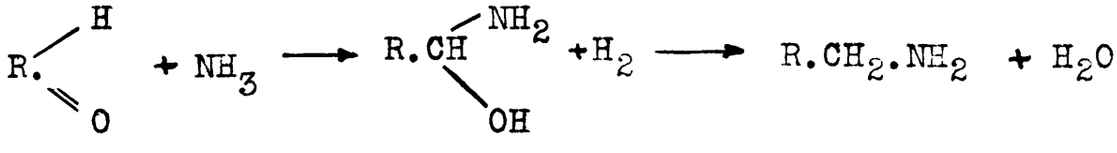
In some interesting experiments, Holtz (1937c) showed that the presence of a catalyst alone would allow of the production of histamine from histidine by means of alternate perfusion of the solution with oxygen and hydrogen. If 20 mg of palladium is added to a solution of histidine and oxygen and hydrogen are alternately bubbled through in a ratio of 30" for oxygen and 90" for hydrogen, histamine will be produced within 30 minutes. If the time relationships are reversed, no histamine is produced.

B. The Action of Ascorbic Acid, Sulphohydrogen Compounds and Animal Tissues on Histidine:

Since histidine is found in the tissues, it is presumed to be one of the main sources of histamine. That the animal organism is able to produce histamine from histidine was demonstrated by Bloch and Pinösch (1936). On the assumption that the transition of the amino acid to the amine occurs by the action of histidase, which is found mostly in the liver, these workers injected histidine into 4 guinea pigs, and after five hours estimated the histamine content of the lungs. According to their findings, the control figures gave values of 15-25 y/gm of tissue. Following the injection of histidine, the histamine content was doubled, i. e. 30-45 y/gm.

In a recent series of articles Holtz (1937a) has described the production of histamine from histidine by the action of ascorbic acid, and compounds containing the S H radicle and certain animal tissues. The assays of the histamine or histamine-like substance were carried out on the anaesthetized atropinized cat. In an effort to explain the possible mechanism of the production of histamine in the organism, Holtz (1937b) has presented the following theories.

(1) The production of amines from aldehydes by synthesis.

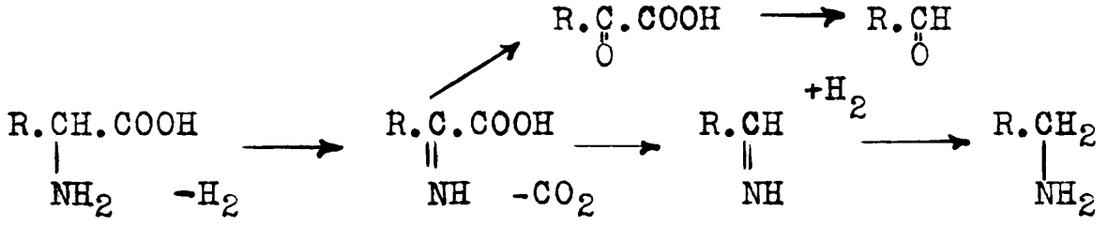


Aldehyde Aldehydeammonia Amine

This scheme is analagous to the synthesis of amino acids from ketone acids and ammonia as shown by Knoop (1910).

(2). Whereas aldehydes form the basis for the synthesis of amines according to the above scheme, it is by decarboxylation of amino acids that amines are more probably formed in the body. According to Holtz the most probable manner of amine formation is by an oxidative deamination process. In the intermediate step an imino acid is formed.

The course of events is represented as follows:



Amino Acid Imino Acid Imin Amine

During the above reaction part of the imino acid is changed to a ketone acid and eventually to an aldehyde, and part is easily decarboxylated to the imine. If at this point a reduction system is present, the imine is easily converted to the amine. The change from imino acid to the amine by decarboxylation can occur as easily as the change from imino acid to amino acid, which occurs in the synthesis of amino acids. It is this change, according to Holtz which determines whether synthesis or break down shall occur. He believes that a reduction oxidation (redoxpotential) system intervenes at such a point.

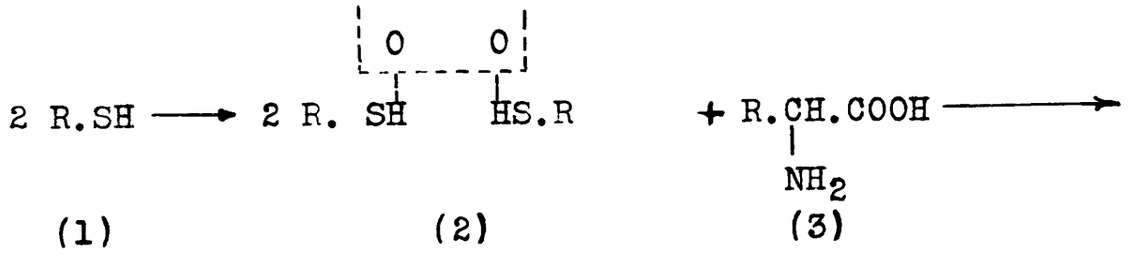
Of the substances essential to cells and tissueswhich are capable of producing such a redox potential system, a variety of reversibly oxidisable sulphur containing compounds such as cystein, gluathione, a few vitamins as ascorbic acid and flavine and also sugar belong. Such systems are greatly influenced by the presence of oxygen. Of particular interest is the work of Vögtlin, Maver and Johnson (1933) who showed that the action of protein ferments is dependent upon the amount of oxygen and reducing substances present, in that under anaerobic conditions or in the presence of reducing substances proteolytic breakdown predominates, whereas in the presence of oxygen or oxidative forms of the redoxpotential system, the protein synthesis action is more prominent. On this basis then, Holtz (1937a) investigated the action of these various substances, cystein etc. on histidine.

It was first shown by Holtz and Heise (1937a) that histamine could be produced from histidine by the action of ascorbic acid or of the sulphydro compounds. Watery solutions of histidine in 1-2% concentration to which either ascorbic acid or cystein was added in a percentage of 0.25-0.5 were first neutralized and then saturated with oxygen. They were then incubated at 40°C for 6-24 hours. Histamine was liberated in small amounts. 200 mg of histidine HCl and 25 mg of cystein yielding 20 y of histamine in 24 hours The reaction between ascorbic acid and histidine is optically specific in that l-ascorbic acid produces more histamine from d-histidine than from l-histidine.

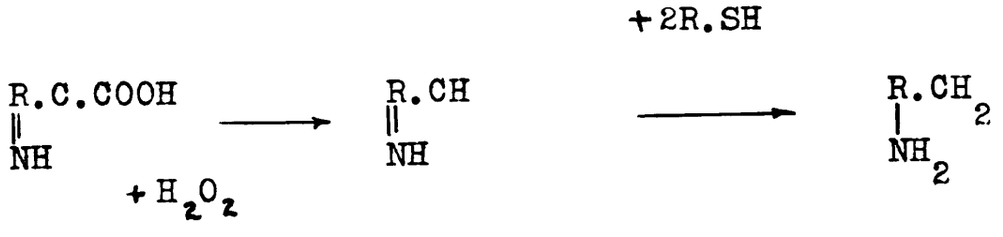
It was noted that ammonia was liberated during the reaction,and it seems probable to these workers that two reactions must take place. 1. decarboxylation; and 2. deamination.

The first is explained by the following reactions. (1) The dehydration of the amino acid to the imino acid and (2) the reduction of the

imine, formed by decarboxylation of the imino acid to the amine. The first step is brought about by the formation of peroxides from the S. H. body (Holtz and Triem 1937) by auto-oxidation. The reduction of the imine to the amine is probably performed by the remaining redox-substance or cystein. The following is Holtz's conception of the above steps.



(1) (2) (3)



(4) (5) (6) (7) (8)

The reduction form of the S. H. compound (1) produces a peroxide like intermediary product under the effect of O_2 (2) which causes dehydration of the histidine (3) to imino acid; (4) The peroxide is then changed back into its original form (5) and at the same time it is oxidised by the hydrogen peroxide to the disulphide form. There then occurs a spontaneous decarboxylation of the imino acid (4) to the imine (6) which by means of the cystein radical (7) is hydrogenized to histamine (8).

In further work Holtz and Heise (1937b) showed that histamine could be produced from histidine by the action of kidney and liver tissue. Glycerin and phosphate extracts of rabbit, guinea pig and rat kidney and liver were incubated for 8-24 hours with watery solution of histidine at 37°C. Toluol was added. The extracts were then filtered and assayed for their histamine content. Since the reaction occurred with ground up tissue, tissue solutions, or tissue extracts, and did not take place if the tissue was first heated to 90°C they concluded that it must be due to a ferment. The reaction will act only in an oxygen free atmosphere, best under nitrogen. They found that 1-2 gms of kidney tissue will produce 200 y of histamine in 12-24 hours and that kidney is 4-5 times more active than liver. It was further established that none of the other tissues which they examined, namely skeletal muscle, heart muscle, lung, spleen, pancreas and duodenum manifested this action.

The effect of pH was studied and it was found that an acid medium inhibited the reaction whereas a neutral or slightly alkaline medium favoured it.

Werle and Herrmann (1937) were able to show that incubation of histidine with rabbit kidney produced a small amount of histamine. On the other hand Zipf and Gebauer (1937) were unable to confirm the results of Werle and Herrmann (1937) and of Holtz. They claimed further that the results of Bloch and Pinösch (1936) were not of significance in that the normal histamine content of the guinea pig lung was as high as in those in which they claimed there was an increase. They also are of the opinion that histidine breakdown depends upon histidine decarboxylase, none of which is present in rabbit kidney.

Influence of Oxygen: - The previous experiments of Holtz and Heise (1937a) (1937b) showed that the production of histamine from histidine was enhanced by an oxygen-free atmosphere, whereas the presence of oxygen inhibited the reaction. This is true in the case of ascorbic acid, S. H. bodies, and tissue extracts. In later work Holtz and Heise (1937c) showed that ascorbic acid was capable of destroying histamine. Realizing that this raised the possibility of ascorbic acid first producing histamine from histidine and then destroying it thereby obscuring the true yield, they ascertained that a minimum amount of the vitamin was essential for the inactivation of the histamine, namely 50 mg. If however, 25 mg of ascorbic acid was added to the histamine (40 y in 10 cc) no destruction took place when it was saturated with oxygen. They then placed this smaller amount ascorbic acid with the same amount of histidine which had been used in the previous experiments and obtained the same yield as had previously been observed. It should be emphasized that production of histamine from histidine takes place only in an oxygen-free atmosphere under the influence of ascorbic acid, at 37°C, whereas inactivation of histamine by ascorbic acid requires oxygen on the other hand.

Holtz and Heise (1937c) further found that the yield of histamine could be increased by augmenting the amount of histidine, keeping the amount of ascorbic acid or cystein constant. If now iron ($FeCl_3$) was added, the production of histamine from histidine was inhibited. They added 0.5 cc $^m/_{100}$ of $FeCl_3$ to 10 cc of a 1-2% solution of histidine to which 25 mg of ascorbic acid had been added. Iron has the same effect on the liberation of histamine by tissues ferments.

C. Bacteria:

In 1910 the direct decarboxylation of histidine by bacterial action was described by Ackerman, and in 1912 Mellanby and Twort described the isolation of a bacillus from intestinal contents which had the power of converting histidine into histamine. It was said to belong to the colon-typhoid group. At this time, many workers more interested in the possible role of histamine in 'intestinal intoxication' and a great deal of work was done, as the power of converting histidine into histamine was attributed to many different types of bacilli. In this earlier work, Ackerman was able to obtain a 40% yield of the theoretical amount.

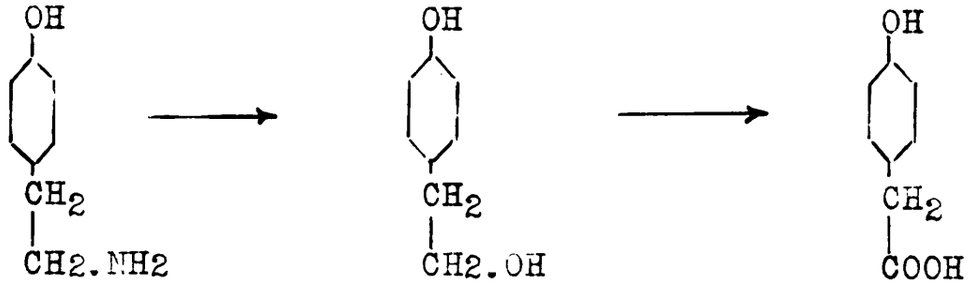
From 1919 to 1924, extensive work was done by Koessler and Hanke and in a later summary of their work (Koessler, Hanke and Sheppard 1928) they discussed the results of the examination of 223 organisms. Of these 94 belonged to the colon-typhoid group out of which 9 converted histidine to histamine. Five out of eight Salmonella morgani produced histamine. The remaining 129 micro-organisms, all of which did not belong to the colon group were not capable of producing histamine. They stated further that they had never observed the production of histamine except in an acid medium. The property of being able to decarboxylate amino acids is therefore not a common one. Among bacteria the organisms capable of doing this are all gram-negative. However a mixture of the organisms commonly found in human feces will generally cause decarboxylation of amino acids, and the decarboxylation is thought to be due to the symbiotic action of the intestinal bacteria.

Bruhl, Ungar and Levillain (1937) reported the production of histamine-like substances by bacterial action on a medium containing nothing but urea. These substances appeared in three to four days.

"The Inactivation of Histamine."

The foundation for the work on the inactivation of histamine was laid down by previous work on the inactivation of tyramine. It was suggested by Ewins and Laidlaw (1910) that the detoxification of this substance occurred in two steps represented as follows:

(1). Replacement of the NH_2 group by OH



(1) (2)

(2). Oxidation of the alcohol to the acid.

Dale and Laidlaw (1911) attempted to inactivate histamine by perfusion through the liver but were unable to demonstrate any inactivation. A suggestion that histamine will be destroyed in the organism was contained in the first observation on the rate of intravenous infusion of histamine by Oehme (1913) and Dale and Laidlaw (1911) who noted that rabbits could tolerate large amounts of histamine intravenously when it was injected slowly, whereas rapid injections caused fatal shock. It was moreover observed that the histamine disappeared rapidly from the blood, and that it was not secreted in the urine.

Eustis (1915) placed minced liver from a buzzard with histamine and incubated the mixture. He found that 10 grams of liver pulp would inactivate 10 mgm of histamine. The inactivating agent was heat labile.

Guggenheim and Loeffler (1916) confirmed the results of Oehme and of Dale and Laidlaw in that they infused large amounts of histamine slowly into rabbits without any deleterious effects.

The fate of histamine in the bowel was studied by several authors, and it was observed that histamine could be absorbed from the intestinal lumen, but that a large amount was inactivated or destroyed either in the lumen itself, or during passage through the intestinal wall. (Mellanby 1915, Meakins and Harington 1922, Koessler and Hanke 1924).

In 1929 the discovery that animal tissue contained an enzyme system which had the property of inactivating histamine was made by Best. An extensive study of this substance by Best and McHenry (1930) revealed the following facts: The greatest activity is shown by the kidney in most species and by the mucosa of the small and large intestine, less by the lung, and relatively slight activity by muscle, spleen and bladder. No such action was found to be present in heart muscle, skin or stomach. The name of 'histaminase' was given to this enzyme system. No excretion of histaminase in the urine was found.

Best and McHenry (1930) further described a method of preparation of a dried powder from ox kidney which could be kept in an active form in air-tight ampoules. The method of preparation of this powder is as follows:

Using beef or dog kidney, 22 kg of fresh material are finely minced and the minced material is placed in a vessel containing 225 litres of 95% acetone. After twelve hours, the acetone is removed by filtration. The residue is washed twice with small amounts of acetone and twice with small amounts of ether and then dried in air. It is then made into a fine powder and stored in air-tight containers.

To prepare an active extract of the substance, the powder is extracted with phosphate buffer at pH 7.0, using 18 parts of the buffer, for one hour with continuous agitation. This yields 50% of the activity. A further 20% may be obtained by a second extraction (McHenry and Gavin 1931). The combined solutions may be concentrated in vacuo at a temperature below 50°C. Saturation with ammonium sulphate precipitates the histaminase. This precipitate is centrifuged after redissolving in distilled water and all the salt remains in the supernatant layer, the precipitate containing the activity. This precipitate may then be ground up with chloroform and again centrifuged. The top layer is drained on hard paper and then air dried. It is redissolved in phosphate buffer and is then ready for use.

The enzyme system has an optimal pH of 6.8-8.0 and optimum temperature of 37°C. The enzyme is heat labile being inactivated above 60°C. Its action is uninfluenced by sodium, potassium or phosphate ions, but it is inhibited by calcium ions, and completely inactivated by potassium cyanide in a 0.0005 molar concentration. This and the fact that oxygen accelerates the reaction suggest that the histamine-histaminase reaction is oxidative in nature. - Best and McHenry concluded that histaminase may cause rupture of the imidazole ring. These authors also performed perfusion experiments and noted that kidney was capable of inactivating large amounts of histamine in comparison with liver or other organs. The relatively small amounts of histaminase in liver explain the failures of the earlier workers (Dale and Laidlaw, Meakins and Harington etc.)

McHenry and Gavin (1932a) showed that a molecule of ammonia was liberated for each molecule of histamine destroyed. This suggests then that the action is one of deamination. It was noted that histaminase was unable to inactivate tyramine.

Further studies on the distribution of histaminase by these authors established that the histaminase content of kidney tissue varies with different species. The results are given in the following table.

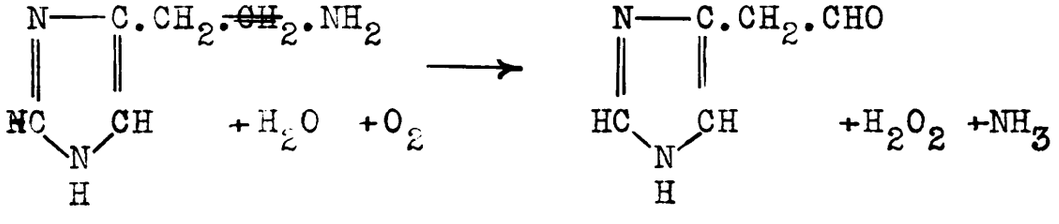
TABLE II

Animal	Mg Histamine Inactivated by 1 mg Kidney
Rat	0
Guinea pig	0
Rabbit	1.6
Dog	1.9
Cat	2.0
Ox	1.6
Hog	3.6
Horse	2.7
Sheep	3.2
Monkey	2.4
Human	0 - 1.6
Chicken	0

Apparently there is no correlation between these figures and the sensitivity of various species to histamine, or of the content of this substance in the various organs of these species. A commercial preparation of histaminase has been prepared by the Winthrop Chemical Company and is

sold under the trade name of 'Torantil'. It is a dried powder made from intestinal mucosa.

Zeller (1937) carried out investigations on the nature of histaminase. He confirmed the observations that the enzymatic histamine splitting reaction is oxidative in nature in that an equivalent of nitrogen is liberated in the form of ammonia. The nature of the breakdown product was established as dinitrophenylhydrazone. During the process, four molecules of oxygen were taken up per molecule of histamine, the first of which liberates a molecule of ammonia. Hydrogen peroxide is formed from the water, as shown by its ability to convert haemoglobin into methemoglobin. The first step is represented by the following:



In further work Zeller (1938a) confirmed the previous observations that histaminase would not inactivate the mono amines such as tyramine, or imidazoles or their compounds with aldehyde, lactic acid or propionic acid, nor histidine or tryptamin. It was not possible to demonstrate a release of ammonia from the action of histaminase on other amines such as adrenalin, amylamin and ephedrin.

He found however (1938b) that oxidative deamination of the diamines, namely ethylenediamin, putressin, cadaverin and agmatin could occur and therefore proposed that histaminase should be called diamin-oxydase, since

the method of breakdown is the same for all of these substances including histamine.

Various perfusion experiments have been carried out in which the rate of destruction of histamine by an organ has been determined. The earlier workers determined that histamine was rapidly inactivated by the blood in the intact animal and that slowly injected histamine would be well tolerated. (Dale and Laidlaw 1910; Oehme 1913; Guggenheim and Loeffler 1916).

Perfusion of isolated organs of the dog has shown that kidney, lung, liver or extremities are capable of inactivating histamine. The heart was not capable of destroying the amine (Best and McHenry 1930; MacGregor and Peat 1933). After perfusion for $\frac{1}{2}$ to $5\frac{1}{2}$ hours the bulk of the histamine has disappeared from the perfusion fluid and cannot be recovered from the tissues.

Since histaminase is abundant in the kidney of most species, it is not surprising that this organ should be the one most capable of destroying histamine when perfused with solutions containing it. MacGregor and Peat (1933) have shown that when a heart-lung preparation from the dog was used, a small but definite amount of histamine was destroyed after one and a half hours. If the kidney is included in the circuit the bulk of the added histamine is destroyed within the first five minutes. In one experiment, 10 mg of histamine was added to the perfusing fluid. It had disappeared entirely within 90 minutes. When the lung and kidney were now examined, the kidney showed no change in its histamine content, whereas the lung showed a slight increase. A small amount was excreted in the urine (0.3 - 1.7 mg).

Essex, Steggerda and Mann (1935) obtained similar results. In their experiments however the kidney was able to remove 25 mgms of histamine from the perfusing fluid within 15 minutes. With the heart lung preparation alone, no such effect was observed. Binet and Marquis (1935) however found that there was a diminution in the blood histamine of the dog after perfusion through the lungs for three hours. They used an indirect method of histamine determination as described by Loeper, Lesure and Thomas (1934) in which imidazol compounds are measured. Sibul (1935) reported that cat's lung removes or destroys 0.3 mg of histamine in ninety minutes.

Dragstedt and Mead (1935) injected histamine intravenously into dogs and assayed the blood and lymph at intervals for their histamine content. When a dose of 0.5 mg/kg was injected giving an initial concentration in the blood of 1:100 000 histamine was detectable in the blood at two minutes but not at five. If the dose was increased to 3.0-4.0 mg/kg traces of histamine were found to be present 30 to 40 minutes after the injection.

Yen and Chang (1933) investigated the anti-histamine activity of defibrinated blood from various species. They defibrinated freshly drawn blood and added varying amounts to 1 mg of histamine. This was then incubated at 37°C for 24 hours under toluol. Rabbit blood showed the greatest activity, 5 cc were capable of completely inactivating this amount of histamine whereas 10 cc of dogs blood was required. Guinea pig blood showed no action at 30 cc and human blood was inactive even in amounts up to 100 cc.

Recently Marcou,(1938) using the method of Barsoum and Gaddum (1935a) for the estimation of histamine has found results which are directly opposed to these. He used whole blood, to which an anti-coagulant had been added (Bayer 205) and incubated in with 30 y of histamine for 30 minutes. It was

found that the blood of pregnant woman during parturition was able to destroy histamine in vitro. This property was found to be present in normal individuals although to a much smaller degree. No histaminase was found to be present in the blood of cats and rabbits, whereas guinea pigs, dogs and human blood displayed it in varying degrees, dog blood having the highest content.

The action of formaldehyde was first investigated by Kendall (1927). He observed that the addition of a small amount of formaldehyde to a bath containing guinea pig uterus inhibited the action of histamine and suggested that this was due to the formation of a condensation product of histamine and formaldehyde. Best and McHenry (1931) investigated this problem further and found that the formaldehyde had a two-fold action. First 50% of the histamine was inactivated immediately. The balance of the histamine was inactivated if kept at room temperature for three weeks. This secondary loss could be speeded up if the solution were kept at 37°C.

Geran (1938) has recently made the interesting observation that solutions of histamine become inactive if they are treated for two minutes with carbondioxide. He used oxygen and CO_2 in a ratio of 20-80. The activity of the histamine was tested upon the guinea pig ileum preparation suspended in Tyrode solution. He noted that changing the pH of the Tyrode solution itself made the gut insensitive to stimulants in general, in that it would not respond to acetyl-choline, $BaCl_2$ or histamine. If however the Tyrode is perfused with CO_2, the preparation will react to acetyl-choline and barium chloride, but not to histamine and therefore he believes that it is a specific test for histamine.

He further observed that histamine solutions inactivated by perfusion with carbon dioxide regained their former potency within thirty minutes if allowed to stand in room atmosphere, or within ten minutes if perfused with oxygen. He believes the mechanism to be due to a reversible carbamino binding.

"The Pharmacological Actions of Histamine."

The pharmacological actions of histamine have been thoroughly worked out by numerous workers, and excellent review of this aspect of the amine are found in the monographs by Feldberg and Schilf (1930); Best and McHenry (1931) and in a more abbreviated form in the monograph by Gaddum and Dale (1936).

A. General Circulation:

Histamine may act in various ways upon the peripheral blood vessels. Under certain conditions it causes a vaso-dilatation, and the resulting drop in the blood pressure is one of its most characteristic effects. Under other conditions it produces a vaso-constriction.

The characteristic and striking drop in blood pressure following upon the intravenous injection of histamine was explained by Dale and Laidlaw (1910) as being dependent upon peripheral vaso-dilatation in the cat. This drop in blood pressure is a constant finding in the cat, dog, man, wood-chuck (Essex 1936) and rat. In the rabbit however the type of anaesthetic is important in that a rise of blood pressure is produced when ether anaesthesia has been used. When other anaesthetics are employed, a drop of blood pressure is observed even in this species. This is important in connection with the identification of histamine, and one should always specify the type of anaesthetic used particularly, in the rabbit.

On isolated strips of arteries and veins, and on the blood vessels of isolated perfused limbs, histamine exercises a constricting action (Dale and Laidlaw 1910) (Dale and Richards 1918).

The vaso-constrictor effect of histamine on the arterioles opposes the vaso-dilator effect on the capillaries, and the ultimate change in the blood pressure depends upon which action predominates. Generally the vaso-dilator effect is the most marked.

Acetyl-choline on the other hand acts mainly by producing vaso-dilatation of the arterioles.

The mechanism of the action of histamine was established by studying the effects of denervation, and of the production of ischemia in the limb of the cat, by plethysmographic methods. If the nerves are left intact, histamine may produce an increase or a decrease in the volume of the extremity, depending upon which of the two above effects predominate. If the limb is completely denervated, both the arterioles and capillaries become dilated; the paw of the extremity is warm and red. In a few moments however the capillaries regain their tone, and the paw becomes blanched, but remains warm due to the dilated arterioles. The action of histamine may now be registered plethysmographically, in that an increase in volume occurs following its injection, due to the capillary dilatation. Acetyl-choline, since it acts by dilating the arterioles, has little effect.

If twenty-four hours is allowed to elapse, the arterioles regain their tone and are constricted, and the vaso-dilator action of both histamine and acetyl-choline is greater.

By the intermittent interruption of the circulation to the perfused cat extremity, varying degrees of ischemia are produced, with subsequent dilatation of both arterioles and capillaries. The arterioles regain their tone faster than do the capillaries. If one now injects acetyl-choline, marked vaso-dilatation occurs, whereas histamine has but little effect.

The vaso-dilator property of histamine can be demonstrated on the isolated cat extremity by perfusion experiments provided the tone of the capillaries is maintained by proper oxygenation. (Dale and Richards 1927). It was shown that as long as capillary tone was maintained histamine had a vaso-dilator reaction. When however the tone was lowered, vaso-constrictor effect on the arterioles predominated. The effect of pH on the capillary tone and on the action of histamine was demonstrated by Hemingway and McDowall (1926). A lowering of the pH from 7.6 to 7.4 caused the capillaries to lose tone, and histamine action on arterioles then again predominated. Change in the response of the blood pressure to the intravenous injection of histamine after altering the pH was observed also by Eichler and Killian (1931).

The action of histamine on the vascular system changes as the biological tree is ascended. Arterial constriction may be the predominant effect in rodents. In the cat there occurs slight constriction of the arterioles and dilatation of the capillaries. Dilatation of both capillaries and arterioles occur in both the dog and the monkey, whereas in man the arterioles and capillaries of the skin and of the brain dilate (Dale 1929).

B. Direct Observations of the Blood Vessels of the Skin:

The first observation on the effect of histamine on the human skin was made by Eppinger (1913) who noted a reddening. It has been observed since by many other workers (Dale and Richards 1918; Lewis and Maruin 1927 etc.).

The so-called 'triple response' which occurs after the introduction of histamine into the skin of human beings has been amply described and

extensively investigated by Lewis (1927). Lewis and Grant (1924) introduced histamine into the skin and made the following observations:

(1) Local reddening: - After twenty seconds a localized reddening occurs which is augmented when the circulation has been stopped, so that its formation is dependent on circulation. If the circulation is normal this effect is not as marked. The reaction is dependent upon the vaso-dilatory action on the capillaries. It can be obtained after degeneration of the local nerves.

(2) Flare: - After another interval of 15-30 seconds, a scarlet flare is produced which has a diameter of 2-3 centimeters. It is dependent upon the presence of intact local nerves and is believed to be due to an axone reflex. If the sensory nerves to the arm are blocked the reaction still occurs but is abolished when the local nerve supply to the skin has degenerated. The reflex is therefore a local one, and does not pass through the central nervous system. The flare is due to arteriolar dilatation.

Use of this reaction has been made for the determination of an intact nerve supply and circulation in patients (Loesser 1938).

(3) Wheal: - The production of a wheal or of localized oedema. This takes longer to appear, usually 3-5 minutes. After this it begins to lose its sharpness and has disappeared at the end of an hour. The mechanism of wheal formation is independent of the nerve supply and depends upon an increased permeability of the capillary wall. There is a marked similarity between the effects produced by the introduction of histamine into the skin and those produced by mechanical or other irritation.

Many observers have confirmed the vaso-dilator action of histamine on various preparations by direct microscopical observation (Hooker 1921; Rich 1921; Grant 1930 etc.).

Action on the blood vessels of different organs: - In general, a vaso-dilatation has been observed in most organs. Dale and Richards (1918) noted such an action on the blood vessels of striated muscle. The vessels of the conjunctiva, brain, intestine, submaxillary gland, pancreas and branches of the hepatic artery all give a vaso-dilator response to histamine (Feldberg and Schilf 1930) (Bauer, Dale, Poulsson and Richards 1932).

The response in the kidney is different however. When the kidney is left in situ, the volume decreases under the influence of histamine (Dale and Laidlaw 1910). In the isolated perfused organ however, a vaso-dilator action was observed by MacGregor and Peat (1933).

The action of histamine on the blood vessels of the brain has been observed in man, and in the cat and dog. In a patient with a traumatic opening of the skull Bruch (1937) observed vaso-dilatation of the cerebral vessels following the intravenous injection of histamine. Its action is influenced by the type of anaesthetic used. Under amytal anaesthesia histamine causes dilatation of the blood vessels of the brain and the C. S. F. pressure rises in man and the cat. This is due to a direct action on the intracranial vessels, and may be an explanation for so-called histamine headache.

In the dog and cat, although there is a dilatation of the vessels, the C. S. F. pressure is lowered by the action of histamine, when the animals are anaesthetized with ether. The fall in C. S. F. pressure occurs at the same time as the general fall in blood pressure and is perhaps dependent upon it. (Lee, 1925; Forbes, Wolff and Cobb, 1929; Pickering, 1933). It was noted by Bruch (1937) that the vaso-dilator action of the cerebral blood vessels occurred before that in the arm. He believes that histamine may be the cause of certain types of syncope, by lowering systemic blood pressure. Bedford (193[illegible])

injected histamine directly into the ventricles without causing any effect on the systemic blood pressure.

C. Action of Histamine on the Heart and on the Coronary, Lung and Liver Circulation.

Heart: - Histamine causes the heart to beat more rapidly and more powerfully when given in small doses. Larger doses weaken the heart, the left side being more affected than the right. (Dale and Laidlaw 1910); Rühl 1929; Feldberg and Schilf 1930). In a recent article Klisiecki and Holdbut (1937) state that the action of histamine on the heart is the primary cause of histamine shock in most animals.

Coronary circulation: - The coronary vessels respond to histamine according to species. The coronary vessels of the cat, dog and turtle heart are dilated, whereas those of the rabbit and guinea pig heart are constricted (Gaddum 1936). Isolated strips of the coronary arteries of man, the ox, horse, pig and dog are contracted. (Cruikshank and Rau 1927; Bürtschi 1937). Cowles and Andrus (1938) noted that the constricting action of histamine on the coronary vessels of the guinea pig heart is inhibited by atropine.

Pulmonary circulation: - The blood vessels of the pulmonary circulation are constricted by histamine in dogs, cats and rabbits (Dale and Laidlaw 1910). Inchley (1923) observed that isolated strips of veins in general, and pulmonary veins in particular contracted when exposed to histamine. Isolated rings of pulmonary vessels contract under the action of histamine. (Feldberg and Schilf 1930). Injections of histamine into the bronchial arteries cause a bronchoconstriction without affecting pulmonary blood pressure, whereas if

injected into the pulmonary circulation, a rise of blood pressure in this circulation and a fall in the general systemic blood pressure is produced. (Alcock, Berry, Daly and Narayana 1936)é

Portal circulation: - The different vascular systems of the liver are acted upon in various ways by histamine. The small branches of the hepatic artery are dilated by histamine whereas the branches of the portal system are slightly constricted (Bauer, Dale, Poulsson and Richards 1932).

However, in the dog, a powerful and important action was noted by Pick and his co-workers. They observed that swelling of the liver accompanied by a marked resistance to the outflow of blood from the hepatic veins into the vena cava took place by the action of histamine (Mautner and Pick 1929). This was called 'Lebersperre' and they believed it to be the underlying mechanism of histamine shock in this species. Bauer, Dale, Poulsson and Edwards (1932) confirmed these observations but were not however able to demonstrate the existence of the same mechanism in either the cat or the goat. Confirmation of Mautner and Pick's (1929) results has also been reported by Tchernogoroff and Popoff (1935).

D. Action on Smooth Muscle:

General: - Histamine causes a contraction of practically all smooth muscle. The uterus and intestine of certain animals are much more sensitive than others and will react to very small doses. Large doses will paralyse both of these organs. Contraction of the bronchi is quite marked, as is that of the retractor penis whereas that of the spleen is only moderately contracted. (Dale and Laidlaw 1910).

None of these actions, including those on the blood vessels is influenced by atropine when it is given in doses large enough to antagonize the action of acetyl-choline. It was noted however that very large doses of atropine will diminish the action of histamine on the intestine and the bronchi (Feldberg 1931) and on the coronary vessels (Cowles and Andrus 1938).

Uterus: - It was shown by Dale and Laidlaw (1910) that the uterus of the virgin guinea pig would react to histamine in doses as small as 1 : 250 million. Pregnancy diminished the sensitivity greatly.

The isolated uteri of cats, dogs or rabbits will also contract when histamine is added to the solution in which they are suspended but they are much less sensitive than virgin guinea pig uterus.

The uterus of the rat responds to histamine by a depression of the spontaneous rhythm. This is not altered after adrenalectomy or by sensitisation (Suden 1934).

Bourne and Burn (1927) injected histamine intramuscularly into a pregnant woman at term. They measured the intrauterine pressure and found that although strong contractions of the uterus were brought about, birth was not hastened.

Intestine: - Dale and Laidlaw (1910) found that isolated strips of intestine were contracted by histamine. In 1916 Guggenheim and Loeffler noted that the terminal part of the guinea pig ileum was very sensitive to histamine, and that it would respond in a quantitative manner to graded doses. The detailed use of such a preparation will be found in a later section on methods. The rectal caecum of the fowl was also found to be particularly sensitive (Barsoum and Gaddum 1935a).

Intravenous doses of histamine in the intact animal produce marked increase of peristaltic movements with later activity of the colon, followed by defecation (Feldberg and Schilf 1930).

Bronchial musculature: - Histamine contracts the bronchial musculature of rabbits, dogs, cats, rats and guinea pigs. The bronchial musculature of the guinea pig is by far the most sensitive of all species to histamine. Foggie (1937) observed that whereas the perfused lungs of the guinea pig responded to doses of 0.1 - 0.2 y of histamine, the smallest dose producing a response in the rat was 5.0 y. Histamine contracts the bronchi of perfused lungs (Bartosch, Feldberg and Nagel 1932; Daly and Schild 1935 etc.) and also of isolated strips of bronchial muscle, but only in much higher concentration (Macht and Ting 1921; Epstein 1932).

Gall bladder: - The musculature of the gall bladder is contracted both when isolated (Halpert and Lewis 1930) and in the intact animal (Ivy and Oldberg 1928).

E. Action on Secretory Glands:

An injection of histamine produces secretion by the salivary, pancreatic and those of the bronchial mucous membrane and tear glands. (Dale and Laidlaw 1910). It also stimulates the gastric glands (Popielski, 1920). It is inactive on the mammary or sweat glands and has little or no action upon the mucous secretion of the intestinal glands. Florey (1930) noted that histamine decreased the secretion of water by the bronchial mucous membrane.

Salivary glands: - The action of histamine on the salivary glands was studied first by Popielski (1920) who noted that part of the secretion was due to chorda tympani stimulation and part due to the release of adrenal by the adrenal glands. A third factor is that due to the contraction of the glands (smooth muscle) (MacKay 1929), and an actual increase in the secretion. The secretion following histamine is increased if the chorda tympani has previously been stimulated.

Gastric secretion: - The ability of histamine to produce a marked secretion of acid gastric juice was noted first by Popielski (1920). This action has been observed in the dog, cat, rabbit, guinea pig, frog (Ketton, Koch and Luckhart 1920) tha rat (Voss 1938) and in man (Carnot, Koskowski and Libert 1922). It will take place when all the nerves to an isolated pouch have degenerated (Ivy and Javois 1924). Gastric juice resulting from the action of histamine is rich in hydrochloric acid but is poor in pepsin. Subcutaneous injections will bring about gastric secretion, but rapid intravenous injections will not. If however, a small amount of histamine is injected intravenously at a slow rate, a secretion of gastric juice ensues (Gutowski 1924).

On the basis that achlorhydria may be due to the constant liberation of histamine from chronic inflammatory skin conditions, Voss (1938) examined the gastric contents of rats before and after chronic histamine injections. Achlorhydria was produced in ten out of twelve rats.

Since histamine is found in relatively large amounts in the gastric mucosa, it has been stated that 'gastrin', the hormone responsible for part of the secretion of the gastric glands is in all probability histamine (Sacks, Ivy, Burgess and Vandolah 1932; Gavin, McHenry and Wilson 1933). Babkin (1934) on the other hand has pointed out a number of difficulties in the way of regarding histamine as the sole agent responsible for the

second phase of gastric secretion. Komarov (1938) however has been able to isolate a substance from the pyloric portion of the stomach which is free of all histamine-like activity, but which has the property of stimulating gastric secretion. Koskowski (1933) has claimed that there is an increase in the blood histamine during the digestion of a meal, but his methods of histamine determination were inadequate. In a recent investigation MacIntosh (1938), using the method of Barsoum and Gaddum (1935a) was not able to show an increase in the systemic blood of the dog during the digestion of a meal. Certain investigations have indicated that histamine may be present in gastric juice (Komarov 1933 and 1936; Brown and Smith 1935). This has also been shown to be the case by MacIntosh (1938), using a method of extraction similar to that described by Barsoum and Gaddum (1935a). He believes that histamine may mediate the secretory action of the vagus on the parietal cells, and that therefore histamine may act as a normal stimulant to gastric secretion.

Action on pancreas: - Histamine exerts a secretory action on the pancreas. (Lim and Schlapp 1922; Molinari Tossatti 1928).

F. Effects of the Administration of Histamine:

(a) Intravenously: - Small intravenous doses of histamine may produce fleeting responses in an animal or in man when given intravenously. Large doses have various effects depending primarily upon the rate of injection and as has been observed in animals, also upon the site of injection. In

general it may be said that the most outstanding and constant response to a small intravenous dose is the drop in blood pressure. It is only natural to suppose then that on increasing the dose of the histamine this effect will be greater. Whether this is so depends upon the species employed and upon other factors. Because of the marked drop in blood pressure, and the shock-like condition produced after an intravenous injection of a large dose of histamine, the earlier workers conceived the idea that liberation of histamine in the organism may be responsible for certain types of shock, in particular traumatic and anaphylactic shock.

If a large enough dose of histamine is injected intravenously into an animal, it causes death of that animal. The mode of death varies in different species. It may be due to one of two actions, (1) the action on smooth muscle as in the guinea pig and rabbit, or (2) on the capillaries as in the cat. The first type of response is usually lessened by anaesthesia, whereas the second is heightened.

Guinea pig: - If an unanaesthetized guinea pig weighing between 200 - 250 gm receives an intravenous injection of 50 y of histamine, a train of typical symptoms ensues followed by death in three to four minutes, due to bronchospasm. The animal first begins to struggle and then to gasp for breath. As the dyspnoeabecomes more marked, cyanosis ensues. The animal urinates, and active peristalsis can be observed through the abdominal wall. The accessory muscles of respiration begin to act and respiration stops, while the heart is still beating. If one opens the chest after death, the lungs are distended and emphysmatous, and the right heart is distended with blood.

Rabbit: - The symptoms of histamine poisoning after an intravenous injection are the same in the rabbit as in the guinea pig. The heart however stops before respiration and death is due to obstruction of the pulmonary circulation, which causes acute dilatation of the right ventricle. The lethal dose of histamine injected intravenously in the rabbit was established by Oehme (1913) to be between 0.6 to 3 mg per kg.

Cat: - The effect of an intravenous injection of histamine was studied in the cat by Dale and Laidlaw (1919). If an anaesthetized cat receives a large intravenous dose (1 mg/kg or more), there is a rapid drop in systemic blood pressure due to the constriction of the pulmonary vessels. This is followed by a secondary rise, which is in turn followed by a gradual sinking of the blood pressure to a low level, from which it does not recover. At this stage, the condition of the animal resembles that of traumatic shock. The fundamental change in histamine shock is a marked decrease in the circulating blood volume, which is due in part to pooling of the blood in the capillary bed, and in part to the transudation of plasma through the capillaries which have become more permeable. This has been shown by the fact that there is a marked concentration of R. B. C. of from 30-50%. Direct estimation of the blood volume has also been carried out in the cat and rabbit by Karady (1938).

Dog: - The general effect of the injection of a large dose of histamine on the dog differs from other animals in that there is produced a marked resistance to the flow or portal blood through the liver which is believed to be due to constriction of the hepatic veins, the so-called 'Venen' or 'Lebersperre'. This work has been carried out mostly by Mautner and Pick (1929). The lethal dose for the dog is given as 3 mg/kg. - In this connection the observations of Jacobs and Mason (1936) are interesting.

A normal female dog received 170 mg per 24 hours intravenously for a period of 62 days (0.066 - 0.7 mg per kg per hour). A total of 2,322 mg of histamine was administered. After 73 days the animal was able to withstand almost ten times the original dose with less signs of prostration than had previously been observed.

Rat: - The rat is very resistant to histamine, and will tolerate large doses intravenously. Voegtlin and Dyer (1924) gave 24.4 mg/kg to a rat weighing 368 gms. and caused a drop of blood pressure from 110 to 42 mm Hg where it remained for a long time. As base this is equivalent to 2.88 mg. Selye (1936) produced an acute phlegmonous appendicitis in rats of the hooded strain by the intravenous injection of 30 mg of histamine hydrochloride. Occasionally, slight ulceration of the stomach was also observed.

Horse: - The effect of intravenously injected histamine was studied in the horse by Ackerblom (1934). If 200 mg of histamine in 100 cc of water is injected, the animal becomes restless before the injection is terminated. The restlessness increases, the mucosa of the eyes, mouth etc. become infected and the pulse is not palpable. Dyspnoea is produced and after another thirty seconds it falls to the ground in a tonic cramp-like condition with the head between the legs. Typical shock is produced with cessation of respiration.

Wood-Chuck: - The blood pressure of the wood-chuck is rapidly lowered by an intravenous injection of histamine (Essex 1936).

(b) Action of histamine when administered subcutaneously or intraperitoneally: - Larger doses are required, when given subcutaneously or intraperitoneally, in order to produce effects similar to those brought about by intravenous administration.

However subcutaneous administration of histamine will produce a marked gastric secretion, whereas a single intravenous injection will not. If histamine is infused intravenously at a slow rate, a secretion of gastric juice does occur (Gutowski 1934).

(c) Enteral administration of histamine: - The effect of enteral administration of histamine depends upon the rate absorption, which in turn varies in different parts of the gastro-intestinal tract. Histamine is most rapidly absorbed from the small intestine. It is very slowly absorbed from the stomach and large bowel. Its action has been investigated by placing a similar dose in various parts of the intestinal tract and observing changes in the blood pressure. The absorption is greatest from the ileum, slightly less from the duodenum. (Meakins and Harington 1923). Much larger doses may be tolerated by the enteral route than by any other.

Histamine is rapidly absorbed from the buccal mucous membrane and a dose which is ineffective when placed in the stomach of a guinea pig may produce death if allowed to come into contact with tongue or mucous membrane of the mouth (Heubner 1925).

Ackerblom showed that histamine would produce symptoms in a horse if it was placed in contact with the mucous membrane of the mouth (1934).

G. Effect of the Chronic Administration of Histamine.

Continuous infusion or repeated injections of histamine may produce different syndromes depending upon the size of the dose, the route, duration of the time of injection, and the species of animal. Changes in the tissues and in the blood have been noted (Henlein 1935; Ackerblom 1934; Ackerblom and Sjoberg 1938).

It has also been shown that pretreatment with histamine will produce a resistance in the organism which may or may not be specific in that it builds resistance against the action of histamine and other nocuous agents. These results will be considered in relation to various pathological conditions in a later section.

H. The Effect of Histamine upon the Composition of the Blood:

(1) Cells: - In dogs and cats, histamine causes a marked concentration of the blood with a corresponding increase of the red blood cells, a diminution of leucocytes and an increase in platelets (Dale and Laidlaw 1910).

The findings on the effect of histamine on the leucocytes are however not uniform. No change could be found in the white blood cell picture after small doses of histamine in the guinea pig (Ahl and Schittenhelm 1913 etc.). Schenk (1922) could observe no change in the white blood cells of man after the subcutaneous injection of 2-5 mg of histamine.

Ackerblom and Sjoberg (1938) working with horses, noted, that the chronic intravenous administration of histamine caused a slight reduction in the red blood cells. The white blood cells on the other hand were markedly increased, both, during and after the injection of histamine. This increase

consisted mainly of neutrophilic leucocytes. A slight decrease in eosinophiles was noted.

(2) Chemical composition:

(a) Blood volume: - Decrease of the total blood volume occurs in cats, dogs (Dale and Laidlaw 1910) and rabbits (Karady 1938). Underhill and Roth (1922) were unable to produce any significant change in the blood volume of rabbits by the use of histamine.

(b) Coagulability:- It is concluded generally that histamine does not affect the coagulability of the blood (Smith 1920; Zunz and La Barre 1926) altough Biedl and Kraus (1912) found that 3 mgm of histamine caused retardation of the coagulation time of the blood.

(c) Chlorides: - The blood chlorides are usually lowered followed the administration of histamine (Drake and Tisdall 1926) (Saradjichvili and Rafflin 1930). No significant change was noted by Jacobs and Mason (1926).

(d) N. P. N.: - According to Hashimoto (1925) and Drake and Tisdall (1926) an increase in the N. P. N. of the blood occurs following the administration of histamine. No change was found after chronic administration of histamine in the dog by Jacobs and Mason (1936).

(e) Protein: - Derer and Steffanuti (1930) observed a decrease in the serum protein of the blood of the cat following histamine administration.

(f) Cholesterol: Blood cholesterol is decreased by an injection of histamine in dogs. (Cornell 1928; Tangl and Recht 1928).

(g) Sugar: - Although varied results have been observed with regard to the effect of an injection of histamine on the blood sugar, it is generally accepted that there is a rise. (Chambers and Thompson 1925; La Barre 1926); (Dzinisch and Pely 1935). This rise in the blood sugar is accompanied by a decrease in the liver glycogen and it was suggested that stimulation of the vagus fibres to the liver might be the cause (La Barre 1926). It was observed however, that although hyperglycemia occurred in the rat following the injection of small amounts of histamine, the injection of similar doses in adrenalectomized rats did not produce any change in the blood sugar with or without transplanted cortical tissue (Wyman and Suden 1934).

In man, the intramuscular injection of histamine did not produce any significant change in the blood sugar (Hiestand and Hall 1932). Similar findings were noted following the intravenous injection of histamine in the horse (Ackerblom and Sjöberg 1938). Large doses of histamine will produce hypoglycemia however. (Katzenelbogen and Abramson 1927; Selye 1938).

(h) Lactic acid: - An increase followed by a decrease in the blood lactic acid was observed following the administration of histamine in dogs by Chambers and Thompson (1925).

(i) Calcium: Varied results have been obtained with regard to the blood calcium. La Barre (1926) found no changes in the plasma calcium of guinea pigs in histamine shock. Kuschinsky (1929) observed an increase of calcium in the serum and plasma of dogs, whereas Ackerblom and Sjöberg (1938) noted a decrease in the blood calcium of the horse.

(j) Phosphate:- Chambers and Thompson (1925) noted an increase in plasma inorganic phosphate in dogs. In guinea pigs however, La Barre (1926)

found the opposite result. Ackerblom and Sjöberg (1938) observed a fall in the horse, which was accompanied by an increase in phosphatase.

(k) Potassium: - A decrease in the blood potassium was noted by Ackerblom and Sjöberg (1938) in the horse.

(l) Lipase: - As measured by Steffanuti (1930) blood lipase was found to be decreased during histamine shock.

(m) Alkali reserve: - The production of both an acidosis and an alkalosis by the administration of histamine has been observed depending upon the mode of injection, subcutaneous injection causing alkalosis and intravenous injection causing acidosis (Wallace and Pellini 1920).

Acidosis however has been produced by the subcutaneous injection of histamine by Hiller (1926), Boyd, Tweedy and Austin (1928) and La Barre (1926).

In the rabbit, Katzenelbogen (1929) produced an alkalosis by intramuscular injections, and Eichler and Killian (1931) produced acidosis by the intravenous injection of histamine.

In the horse, variable results were obtained (Ackerblom and Sjöberg 1938). In man no change in the alkali reserve was observed following the intravenous injection of histamine (Weiss, Robb and Ellis 1932) whereas only a slight increase of the CO_2 combining power of the plasma occurred following intramuscular injections (Hiestand and Hall 1932).

"The Effect of Histamine on Lymph."

Dale and Laidlaw (1911) observed that an injection of 2 mg of histamine produced an increase in the flow of the lymph, from 0.3 cc to 1 cc per minute in the thoracic duct of the dog. This action is thought to be due to an increase of lymph from the liver because the constriction of the liver veins. Haynes (1932) noted an increase in the lymph flow from the leg of the dog following an injection of histamine.

"The Action of Histamine in Man."

(a) Subcutaneously: - Many observations have been made on man following the subcutaneous injection of histamine in doses of from 0.5 - 8 mg (Kehrer 1912; Schenk 1921 etc.). It produces flushing, particularly about the face and neck, quickening of the pulse and pounding of the heart, nausea, occasionally asthmatic-like symptoms, and is accompanied by a marked fall in blood pressure. Secretion of acid gastric juice occurs. If the dose is increased, the symptoms are more severe, and an acute headache, throbbing in nature may be produced.

Recently Parrot (1938) has noted that patients suffering from asthma respond in a different manner to subcutaneous injections of histamine. The blood pressure rises instead of falling.

In patients with migraine however, a steep fall of the blood pressure was noted, followed by a slow rise and eventual hypertension, at which point the headache is observed (Parrot 1938).

(b) Intravenous Injection:- The intravenous injection of histamine has been studied by Weiss and his co-workers in particular. Weiss, Robb and Blumgart (1929) gave injections of 0.001 mg (1 y) per kg of the phosphate and measured the circulation time by noting the appearance of flushing about the face and neck, and by the metallic taste produced in the mouth. The average dose given was 60-70 y of the phosphate which is equal to 20-25 y of histamine base. In another paper Weiss, Ellis and Robb (1929) described the changes appearing in patients during the continuous venous infusion of 0.0003 mgm of the amine per minute. In some adults 0.02 - 0.04 mgm per minute were injected. They studied cardiac output and rate, blood velocity and pulmonary ventilation all of which were increased. Basal metabolic rate was also increased. Dilatation of the peripheral arterioles, capillaries and venules was observed. The blood volume was not changed but the distribution of blood was shifted so that that on the venous side was increased. No fall in blood pressure could be noted at an injection rate of 0.02 mgm per minute, and they came to the conclusion that the resulting condition in no way resembled shock.

Further studies on the effects of a rapid injection of 0.05 - 0.08 mgm of histamine intravenously as the phosphate (0.016 - 0.026 mgm base) were carried out by Weiss, Robb and Ellis (1932). The general conclusion arrived at was that the production of symptoms depended upon the rate of injection as well as on the size of the dose. They found that there was an inversion of the T. waves in the E. C. G. taken during the time of injection. After the injection of 20 y they were unable to note any marked changes in the B. P.; a rise of about 15 mm Hg occurring in some and a fall of about the same magnitude in others. Weiss, Robb and Ellis (1932) state 'the most significant of these findings is that man does not as a rule respond to histamine with a prompt fall in arterial pressure.'

Further observations of Weiss et al (1932) showed no change in venous pressure. Measurement of the C. S. F. pressure showed a rise which was not accompanied by an increase in the arterial pressure. This observation was made both under ordinary conditions and at operation. They consider the vessels of the brain to be the most sensitive of all the vessels in the body to histamine, and state that there is a progressive decrease in the sensitivity of the cutaneous vessels from head to foot. No changes during injection of histamine were noted in the nail bed capillaries as observed under a special microscope. In further observations on continuous infusion of histamine, they found that symptoms could be varied according to the rate of infusion. Increasing the rate of injection augmented the severity of symptoms and vice versa. Sudden changes in concentration play an important part in precipitating headaches. They state 'these observations clearly demonstrate that histamine is changed to a pharmacologically ineffective substance or substances practically as fast as it is infused into the human circulation.'

In an effort to determine the sensitivity of man to histamine, Karady (1934) injected small doses of histamine (5 y of the base) intravenously. By following the systolic blood pressure, marked responses to such a small dose was observed in certain individuals, and he was able to distinguish four types of responses. Type I is characterized by a drop in the systolic blood pressure of about 20-30 mm Hg within 30 seconds after the injection, followed by a return to the normal level within one and a half minutes. Type II is characterized by an initial drop of at least 20-30 mm Hg followed by a return to the original level and a secondary rise of at least 20 mm Hg above the initial level with a return to the normal B. P. finally. To the

third type belongs that group of individuals whose blood pressure hardly varies, a fall of 5-10 mm Hg with a return to normal. Type IV consists of a primary fall of the blood pressure of less than 20 mm Hg followed by a return to the normal and a secondary rise of less than 20 mm Hg above the normal level, with a final return to normal. In later studies Rusnyak, Karady and Szabo (1936) studied 320 patients with this method and noted that 258 fell into the first group (Type I), 30 to Type II, 14 to Type III, and 18 to Type IV. An analysis of the fate of all of these patients showed that those belonging to the second type tended to go into surgical shock if subjected to severe operation, whereas none of the others manifested this tendency. Further discussion of these findings will be found in a later section.

It is interesting in this connection to consider the observations of Barsoum and Smirk (1936a) who determined the blood histamine in patients in congestive heart failure. They found an increase in the total blood histamine which varied from 0.08 - 0.3 y/cc (normal 0.04 y/cc). Practically all of this histamine was held in the cellular elements with a small amount only in the plasma. That the combined histamine was inactive was demonstrated by the fact that a small amount (7-10 y) injected intravenously into such patients produced the same effect as in a normal patient, in other words, this injected histamine was free to act in spite of the fact that much higher concentrations were present in combination with the cells.

"The Extraction and Identification of Histamine."

In the introductory sections of this work, it was noted that since histamine may be confused with many other similarly acting substances, great care must be exercised in its extraction, and adequate proof that such an extracted substance is histamine must be adduced. Again, since the amine is so active pharmacologically in very small amounts and frequently is present in minute quantities, the difficulty of separating it from other tissue hormones will readily be seen.

There are various methods in use for the extraction of histamine. It seems to be quite stable in tissues since finely minced tissue may stand for a few hours at room temperature, without either a production or destruction of histamine taking place as far as can be determined with present methods. The controversy as to whether histamine exists as the free substance in loose combination, or in the form of some precursor such as a decarboxylated form of histidine has not yet been established, although it is known that histamine can readily be formed by the action of various substances and tissues under the proper conditions, and similarly destruction can take place. Because of this, and the fact that bacterial invasion readily alters the histamine content of tissues or extracts, one should perform the extraction on as fresh tissue as possible.

Since the methods for the chemical isolation of histamine are very lengthy and involve considerable loss of the substance with each step of the procedure, biological methods have been resorted to by most workers.

Methods of Extraction.

Tissues:

(a) - Finely ground up tissue is placed in 95% ethyl alcohol (2-4 cc/gram of tissue). This is filtered after allowing to stand 24 hours. The tissue is again extracted with 60% alcohol for 24 hours and then filtered. Both extracts are mixed and the volume reduced in vacuo. Fat is removed by ether which also removes choline and adenosine and the final extract is then evaporated to dryness in vacuo, the residue is taken up in salt solution and tested biologically.

This method was used extensively by earlier workers. Best and McHenry (1931) demonstrated that the first two extractions with alcohol yielded 60% and 30% of the histamine respectively and recommended a third extraction.

(b) - In their work on methods for the determination of histamine, Koessler and Hanke (1920) made the important and useful observation that histamine was stable when subjected to acid hydrolysis.

Taking advantage of this fact, Best and McHenry (1931) introduced a new method in which tissue is placed directly into 10% HCl (150 cc of acid for 20 gm of tissue) and heated to 95°C for one hour, after which time the tissue has disintegrated. This mixture is then dried in vacuo and washed twice with 50 cc portions of 95% ethyl alcohol. The residue is then taken up in distilled water and neutralized with 20% NaCl. This is then filtered and the filtrate is assayed biologically. The residue should be thoroughly washed in order to obtain all the histamine in the filtrate. This method has been used extensively.

(c) - MacGregor and Thorpe (1933) have described a method by which histamine may be electrodialysed from tissue mixtures. A cell containing three chambers separated one from the other by collodion, is used. The tissue mixture is placed in the centre chamber. The other two contain the anode (carbon) and cathode (nickel). A current of 0.2 - 0.5 amperes is used, which attracts the histamine to the cathode. After a suitable time, determined by testing the fluid by means of the Pauly reaction every fifteen minutes (two similar results indicating that dialysis is complete), the contents of the positive chamber are removed for biological assay. The solution must be kept cold.

Since this procedure requires but two hours at the most, it is much shorter than the two described previously, and although it gives results comparative with method a , the yield is 30% lower than that of method b. (Gaddum and Dale 1936).

(d) - The method for the extraction of histamine from blood described by Barsoum and Gaddum (1935a) has been used considerably in recent years for extracting histamine from tissues. In general the following procedure is used (as modified by Code 1937b): The weighed tissue is finely ground up with sand and placed in 10% trichloracetic acid (2 gm of tissue to 15 cc). After standing about one hour, this is then filtered, and the residue washed several times with 10% trichloracetic acid. The filtrate is then boiled for an hour with 5 cc concentrated HCl. This then evaporated down in vacuo and washed with two 10 cc portions of 96% alcohol. The residue is then taken up in distilled water, neutralized and assayed on the guinea pig ileum preparation.

(e) - In recent work Feldberg and Kellaway (1937) used a simple method for the extraction of histamine from the lungs of cats and guinea pigs. The tissue was ground up finely in Tyrode solution in a mortar. After thorough grinding, the whole was made up to so that 20 - 50% equalled 1 gm of tissue. The solution was then brought to the boiling point, and after cooling, was tested on the guinea pig ileum preparation. They obtained results comparable to those found by the more complicated methods described above.

Methods for whole blood:

(a) Barsoum and Gaddum: - The original method of Barsoum and Gaddum (1935a) has been used for most of the work by many observers. It is as follows:

Ten cc of blood are run into 15 cc of 10% trichloracetic acid. After standing one hour, it is filtered and the residue is washed four times with 5 cc trichloracetic acid. The filtrate is shaken four or five times with successive portions of ether in a separating funnel until it is about neutral to congo red paper. Ten cc of N HCl are then added and the whole is boiled for 90 minutes in a flask, water being added if necessary to prevent desiccation. The extract is then evaporated to dryness in vacuo, and the excess HCl removed by the addition of two 10 cc portions of 96% alcohol.

The residue is extracted three times (2 x 2 x 2 cc) of alcohol previously saturated with NaCl. The alcoholic extract is filtered and evaporated to dryness, and the residue is taken up in 2 cc of water neutralized to litmus with x cc of N NaOH (0.28 - x cc) of N NaCl is added and the total volume is made up to 2.5 cc.

(b) Code: - The method of Barsoum and Gaddum was studied by Code (1937b) and it was noted that the results on two samples of the same blood might vary considerably. He also noted that the ether extraction was unnecessary, and modified the method as follows: 10 cc of blood are run into 15 cc of 10% trichloracetic acid, and are thoroughly mixed to precipitate the proteins. After standing for approximately one hour (30 - 90 minutes) this is filtered by suction and the precipitate is washed four times with 5 cc of the trichloracetic acid. Ten cc of concentrated HCl is added to the filtrate and the mixture is gently boiled for 90 minutes using a reflux air condenser to prevent too rapid evaporation. After this hydrolysis the mixture is evaporated in vacuo, and the residue is washed with two 10 cc portions of absolute alcohol. The dry residue is taken up in 2 cc of distilled water and after allowing it to stand for a few minutes the whole is filtered. The flask containing the dry residue is again washed with two more 2 cc portions of water, and these are also filtered. The filtrate is neutralized with $^{N}/_{5}$ NaOH using brom-thymol-blue as an indicator. About 1.0 - 1.5 cc of $^{N}/_{5}$ NaOH is all that should be required. The extract is now made up to the original volume of the blood sample taken and is ready for assay on the guinea pig ileum preparation.

(c) Method for differential blood histamine: - If the source of blood is sufficient to provide larger quantities, in the neighbourhood of 30-40 cc, histamine determination of the plasma, red or white blood cells may be made.

The blood must be drawn quickly in a paraffined syringe and oiled needle. It is placed into paraffined centrifuge tubes standing in ice. Heparin is the only anti coagulant which may be used.

Various parts of the blood may be removed after centrifuging, i. e. plasma, red blood cells or the 'white blood cell layer'. The method of

extraction and assay is as described above.

(d) - Tarras-Wahlberg (1935) described a method for the detection of histamine in the blood plasma of the rabbit. Freshly drawn blood was centrifuged and the plasma poured into collodion caps. The plasma containing caps were then immersed in cold water. The plasma was dialysed against 5-6 times its volume of water. After changing the water about the collodion sack 2 - 3 times, the separate portions were mixed into one and evaporated to dryness in vacuo. After several treatments with 99% alcohol to remove excess mineral salts, the final residue was taken up in normal saline and assayed biologically. He obtained a value of 0.5 y/cc of plasma in the blood of the rabbit, which is slightly higher than the figure obtained for plasma histamine in this animal by Code (1937c).

"The Identification and Estimation of Histamine."

There are two ways of identifying and estimating histamine, apart from the actual chemical isolation. These are first chemical, and secondly biological.

(1) Chemical Methods:

(a) In 1904 Pauly described a test for the identification of the imidazol nucleus. It depends upon the formation of coloured compounds originating from the combination of the imidazol ring of histamine with diazo substances. In the original method, potassium nitrite was added to a solution of sulphanilic acid in hydrochloric acid, in order to form diazobenzolsulphonic acid. Sodium carbonate was then added to the solution to be investigated, making it strongly alkaline, and the diazo solution, prepared as indicated above, was then added. The production of a deep cherry red colour occurs in a positive reaction. Addition of acid changes the colour to orange. The reaction is not specific for histamine, since histidine gives a similar result. Tyrosine produces a red colour which however changes to bronze with the addition of acid.

Many workers have made use of this reaction. Koessler and Hanke (1919) worked out a quantitative chemical method in which the final determination was made by use of the Pauly reaction. They were able to estimate solutions containing 1 - 50 y. By means of a lengthy and complicated chemical extraction, a relatively pure extract of histamine was obtained. However, there were many objections to their method, the most important one being that great losses of the amine were encountered during the process of purification (Gemard 1922; Best, Dale, Dudley and Thorpe 1927). In spite of this fact, the method might

be of greater value, were it not that biological methods are so much more sensitive and simple.

The Pauly reaction was modified by Gebauer - Fuellnegg (1930). He substituted p-nitraniline for the diazobenzolsulphonic acid. In this way it is possible to extract the colour compound due to histamine or histidine with butyl alcohol.

Yokoyama (1936) has described a further modification which is micro-colorimetric in nature. Using this method, histamine was found in the blood of the rabbit but not in that of the dog.

(b) Knoop (1908) described a test for histamine and histidine. Both gave a reddish brown colour when placed in a solution of bromine. The test was modified by Hunter (1922). It is however less sensitive than the Pauly reaction and is not quantitative.

(c) A specific test was described by Zimmerman (1929). When histamine is placed in a Thunberg tube in vacuo, and is mixed with cobalt nitrate and alkali, a violet colour is produced. It is however not a sensitive test since it will detect only quantities of 0.5 mgm or over.

(2) Biological Methods:

As no one of the biological methods for the identification of histamine is specific in itself, one must use as many tests as possible to ascertain that the substance is histamine and not one of the other tissue substances.

In general, choline and acetyl-choline may be ruled out by the use of atropine, but this should be used only when necessary, since atropine may lessen the sensitivity of a preparation to histamine.

Adenosine derivatives, kallikrein and 'substance P' have histamine-like actions, but these are destroyed by acid hydrolysis. Table III shows the principle distinguishing properties (Ungar 1937).

TABLE III.

THE CHEMICAL AND PHARMACOLOGICAL CHARACTERISTICS OF THE PRINCIPLE TISSUE VASO - DILATOR SUBSTANCES.

SUBSTANCE	Solubility in Alcohol	Precipited by: Phosphotungstic acid	Precipited by: Trichloracetic acid	Resists 100° C.: Acids	Resists 100° C.: Bases	Deamination	Dialysis	Adsorption on charcoal	Inactivation	Pauly Reaction	Arterial Pressure: Cat, Dog	Arterial Pressure: Rabbit	Heart: Chronotropic action	Heart: Inotropic action	Cutaneous Reaction	Gastric Secretion	Urinary Secretion	Action on Isolated Uterus: Dog, Cat	Action on Isolated Uterus: Rabbit Guinea-pig	Action on Isolated Uterus: Rat, Mouse	Isolated Intestine: Dog	Isolated Intestine: Guinea-pig	Action on muscle of the leech	Atropine	Eserine	Cocaine	Sympathetic inhibit.agents	Smallest detectable dose
Acetyl-choline	+	+	0	+	0		+	+	blood esterase		-	-	-	-	0	0+	+	+	+	+	+	+	+	-	+	0	0	0,001 y
Choline	+	+	0	+			+		0	0	0	0	-	0			+	0+	0+	0+	0+	0+	+	-	0			100 y
Adenylic Acid	0+	+	0	0	+	+	+			0	-	-	-	-				-	+	-	-	-		0	0			
Histamine	+	+	0	+	0	+	+	+	Histaminase	+	-	+	+	+	+	+	-	+	+	0	+	+	0	0	0	0	-	0.01 y
Kallikrein	0	+	+	0	0		0	+	blood	+	-	-	+	+		+	+	-	+		+	+	0	0	0	+		0.004 y
Substance P	+	+	0	0	0	0	+	+			-	-			0									0				

(a) Blood pressure: - The blood pressure of the cat is one of the most generally used indices. It is sensitive, and quantitative in amounts from 0.5 y up. It is dependent upon the fact that small intravenous injections of histamine produce a rapid fall in blood pressure. The animal may be anaesthetized either with ether or most of the standard anaesthetics (Dial, nembutal, urethane and chloralose etc). Injections are made regularly every three minutes, stopping the kymograph in between to facilitate comparison. Alternating doses of the extract and a standard of histamine varying from 0.3 to 1.0 y are injected until the strength of the unknown is determined. The carotid or femoral artery may be used. This method is described in detail by Burn (1928).

The dog is sometimes used for histamine assay but it is much less sensitive than the cat.

When identification rather than quantitative assay is required, the rabbit may also be used. It is important to remember that in this species the type of anaesthetic used may alter the response of the blood pressure to an intravenous injection of histamine. Under ether anaesthesia a rise of blood pressure is observed whereas under other anaesthetics usually no response or a definite fall may occur.

(b) Uterus: - According to Burn and Dale (1922) the uterus of the virgin guinea pig may be used as a quantitative test for histamine. The method was first described by Dale and Laidlaw (1910). The uterus contracting activity of the unknown extract is compared with that of known histamine standard solutions. The test is however not specific since many other substances including posterior pituitary extract, choline derivatives and adenosine compounds will also cause contraction of the uterine muscle.

Uteri from other animals are much less sensitive and react in different ways to histamine.

(c) Intestine: - One of the most useful and convenient methods of assaying histamine is by means of the guinea pig ileum preparation. This was first described by Guggenheim and Loeffler (1916). It is one of the most sensitive of preparations and allows of comparatively rapid assay in that the gut relaxes quickly when the histamine solution is washed away, and it is thus ready for a stimulus every ninety seconds. A good preparation moreover may be constantly used for as long as two hours. Details of this method will be found in the description of the experimental work.

Choline and its esters cause guinea pig intestine to contract whereas adenosine and its derivatives cause relaxation. Atropine will inhibit the action of choline or acetyl-choline, but large doses also diminish the sensitivity of this preparation to histamine.

Such a preparation will react to 0.01 y with accuracy and will distinguish increments of 0.005 y of histamine.

Intestine of other animals such as the cat and rabbit have also been used, but they are much less sensitive to histamine than that of the guinea pig.

Recently Barsoum and Gaddum (1935a) have used the rectal-caecum of the fowl for the determination of histamine. This is the most sensitive of all known biological preparations.

It should be noted at this point however that the guinea pig ileum preparation may be affected by various other substances other than the tissue vaso-dilators such as histamine, acetyl-choline and adenosine. This is of particular importance in the assay of histamine from perfused lung preparations. For instance, snake-venom, bee-venom and staphylococcus toxin all of which cause the liberation of histamine, also produce a peculiar type of contraction when they are allowed to act on the gut preparation. In distinction to these substances, which produce slow contraction and delayed relaxation

following the washing out of the bath, histamine produces a rapid contraction of the intestine, following by practically immediate relaxation, when the bath is washed out. Furthermore, the intestine becomes refractory to small doses of these other substances, whereas it continues to give the same approximate response to repeated small doses of histamine. (Feldberg and Kellaway 1937; Feldberg and O'Connor 1937; Feldberg and Krogh 1937; Rocha E Silva 1938).

(d) Bronchi: - Various methods have been described for the registration of the broncho-constriction which follows upon the action of histamine when given intravenously in the guinea pig.

Koessler, Lewis and Walker (1927) described a method whereby the action of 5 y of histamine could be detected. Other techniques have been worked out by Thornton (1931), Bartosch, Feldberg and Nagel (1932) and Daly and Schild (1935).

(e) Gastric secretion: - Popielski (1920) noted that 3.3 y/kg body weight of histamine produced gastric secretion in the dog. This method has been used extensively by Ungar and his co-workers (1937), who claim that no other substance cause this action in such small doses. One hundred times this amount of acetyl-choline is required to produce the same effect, and the action of acetyl-choline may be inhibited by atropine. (Gebhart and Klein 1933). Recently however it has been noted that kallikrein will stimulate gastric secretion (Ungar 1937).

(f) Adrenal glands: - Histamine has the property of releasing or causing a secretion of adrenalin from the medulla of the adrenal gland. This was noted by Burn and Dale (1926) and Szczygielski (1932).

(g) Human skin: - If the skin is pierced by plunging a needle through

it at the site where a drop of histamine has been placed, a typical response is obtained as has been described above.

"The Chemical Isolation of Histamine."

Since this thesis is concerned mainly with the properties of histamine, and in particular with its metabolism, a review of the chemical methods of isolation would be out of place. Suffice it to say that the amine has been isolated usually as the picrate from intestinal mucosa by Barger and Dale (1911), from gastric mucosa by Abel and Kubota (1919), and Sach, Ivy, Burgess and Vandolah (1932), from hypophysis by Abel and Kubota (1919), from lung and liver by Best, Dale, Dudley and Thorpe (1927), from striated muscle by Thorpe (1928), from spleen by Dale and Dudley (1929), from heart by Thorpe (1930) and finally from blood by Code and Ing (1937).

"The Liberation of Histamine under Various Conditions."

Although the word histamine is used mostly throughout this section it must be remembered that in some of the work presented, the authors refer to histamine-like substance, or 'H' substance. Methods of identification will only be referred to as they have already been discussed.

(1) The Liberation of Histamine-Like Substances by Nerve Stimulation: -

The work on this aspect of histamine liberation has been carried out for the most part by Ungar (1937) and his collaborators. They noted that during the antidromic stimulation of sensory nerves, and following the vaso-dilator response by means of the sensory (peripheral) axone-reflex, a histamine-like substance was liberated into the circulation. Their criterion for this was based upon a secretion of the gastric juice, following stimulation (antidromic) of various nerves.

In spite of their work, there is still some dispute as to what the active vaso-dilator substance really is. Wybauw (1936) believes it to be acetyl-choline, whereas Kibjakow (1931) is of the opinion that it is neither a histamine or acetyl-choline like substance. In some of their recent work Ungar, Parrot and Grossiord (1936) have produced evidence that it may be kallikrein, since they found kallikrein in the saliva of a dog following stimulation of the lingual nerve and further that kallikrein may stimulate gastric secretion (Ungar and Parrot 1936).

Ungar (1935) was able to produce gastric secretion in dogs by stimulation of the peripheral end of the cut crural nerve. Stimulation of the central end, or of either end of a cut motor nerve was ineffective. Atropine did not inhibit this action (Ungar, Zerling and Pocoulé 1935).

Therefore the substance liberated could not have been acetyl-choline. Ungar and Zerling (1935) produced a secretion of gastric juice by reflex stimulation, i. e. stimulation of either the pressor or depressor nerves. It is known that stimulation of either the nerves from the aortic arch, or those from the carotid sinus produce a drop in blood pressure ~~by a drop in blood pressure~~ by a general reflex vaso-dilatation. (Bayliss 1908; Tournade and Malmejac 1933). These experiments were carried out on dogs with crossed circulation by anostomosis of the carotid and jugulars. Gastric pouches were also prepared. The depressor nerve, or the vagus was stimulated in dog A. This was followed by a lowering of the blood pressure, and a secretion of gastric juice in this animal. The second animal B, connected by circulation only showed a secretion of gastric juice also. Both animals were atropinized. It was also shown that placing of a dog's paw in water at 50°C would produce a gastric secretion in that animal (Tinel and Ungar 1935). They believed that the liberation of the active substance in this instance was due to local axone-reflexes of the sensory nerves since following the degeneration of sensory nerves after cutting no secretion was observed.

Further experiments on the release of a histamine-like substance from organs by the stimulation of nerves was performed. First it was shown that the production of emboli in lungs and bowel infarcts would lead to a secretion of gastric juice in the dog. (Ungar, Contiades and Palmer 1935; Ungar, Grossiord and Brincourt 1935). These lesions were produced by the injection of lycopodium. It was further shown that injections of histamine into the mesenteric artery or stimulation of the peripheral end of the cut splanchnic nerve would reproduce these lesions as well as the secretion of gastric juice. Cross circulation experiments were also performed as previously described, and a secretion of gastric juice in the second animal was always observed. (Ungar, Contiades and Grossiord 1935; Ungar, Grossiord and Brincourt 1935). It was

thought therefore that the local liberation of histamine at the site of the lesions was responsible for their production. Injection of lycopodium caused local axone-reflexes from sensory nerves, or by direct antidromic stimulation of the cut splanchnic nerve with the production of axone-reflexes and liberation of histamine.

In further investigations, Ungar, Grossiord, Brincourt and Parrot (1935) showed that certain branches of the phrenic were responsible for the liberation of the histamine-like substance. They therefore proposed calling them "histaminergic".

In this connection it should be noted that MacGregor and Peat (1931) were unable to show any change in the histamine content of cats lungs following stimulation of the phrenic. Riesser (1937) however noted an increase in the blood of the guinea pig following stimulation of the vagus nerve.

Ungar, Parrot and Grossiord (1936) were able to produce gastric secretion in the atropinized dog by stimulation of the lingual nerve, whereas if the animal were not atropinized, no secretion occurred. This was explained by the fact that fibres of the chorda tympani would release a histamine-like substance following stimulation. In the atropinized animal, this substance is liberated into the circulation and causes a secretion of gastric juice. In the non-atropinized animal however, it is secreted in the saliva, and gastric secretion does not occur. Evidence has been put forward to show that the vaso-dilator substance secreted in the saliva by stimulation of the chorda tympani, however is not histamine (Feldberg and Guimarais 1935). Ungar and Parrot (1936) have shown that gastric secretion may be produced bykallikrein, and they believe it to be the substance responsible for this action following stimulation of the lingual nerve. In reference to this Feldberg (1937) makes the comment that all of the other findings of Ungar and his co-workers, i. e. production of gastric secretion following nerve stimulation, may be due to a release of

kallikrein as well, and that one may perhaps speak of "kallikreinergic" nerves.

Other workers have shown that substances such as acetyl-choline (Dale and Gaddum 1930) or vaso-dilator substances of unknown constitution may also be released by the antidromic stimulation of nerves (Kibjakow 1931) The observations of Ungar (1937) are therefore still open to question.

(2) The Release of Histamine during Metabolic Activity:

(a) Circulatory change, oxygen want, or CO_2 increase: - Disturbances of circulation, oxygen want, acidosis and other similar conditions are all closely allied with reactive hyperemia and lead to an increase in the amount of demonstrable histamine in the blood. The method used in the extraction of the histamine in most experiments has been that of Barsoum and Gaddum (1935a) or the modification of it by Code (1937b).

Barsoum and Gaddum (1935b) showed that interference with oxygenation of the tissues by interference with blood supply or by a deficient amount of oxygen and also by the presence of an excess CO_2 content all produced an increase in the histamine content of the venous blood coming from the limb of an animal. They believe that release of histamine is in part responsible for some of the phenomena of reactive hyperemia, and the experiments with carbon dioxide suggest that the release of histamine is a secondary consequence of the accumulation of acid metabolites.

Anrep and Barsoum (1935) found no difference in the histamine content of the arterial and venous blood flowing through resting muscle. It was noted that histamine was increased in the venous blood when the perfusion pressure was lowered. They used the perfused gastrocnemius muscle preparation of the dog as described by Anrep and von Saalfeld (1935).

Using rabbits, Tarras-Wahlberg (1936) was able to show that venous blood possessed a histamine-like action, whereas arterial blood did not. He took blood directly by cardiac puncture and assayed it on guinea pig ileum. This histamine-like activity of the venous blood was increased by asphyxia and by haemorrhage. He extracted both plasma and whole blood by the method of Barsoum and Gaddum (1935a) and observed that whereas a marked increase of the plasma occurred after haemorrhage, the total blood histamine was not altered. He therefore speaks of the 'free' and 'bound' histamine of the blood. These results are better explained by the work of Code (1937d) who demonstrated that the source of the blood histamine in the rabbit and other animals is the white blood cells. Therefore with destruction of these cells, histamine is liberated into the blood plasma. Tarras-Wahlberg (1936) showed further that the histamine content of the lungs increased following asphyxia in the cat and rabbit. He used one lung as a control in each case.

Barsoum and Smirk (1936) examined the blood of normal individuals and patients with congestive heart failure. Whereas the histamine content of the blood of normal individuals varied between 0.05 - 0.11 y/cc, that of the blood of patients with heart failure varied from 0.08 - 0.3 y/cc. They found further that this increase occurred in the cellular elements, the

histamine content of the plasma being the same in both groups. They used the method of Barsoum and Gaddum (1935a) for the extraction of histamine.

On the other hand the production of reactive hyperemia in normal individuals was followed by an increase in the plasma histamine. Since the histamine was increased in the plasma in these cases, it was free to produce its effects, that is to cause vaso-dilatation. In the cases of heart failure on the other hand the excess histamine was bound to the cells and was therefore inactive (Barsoum and Smirk 1936).

The observations of Eichler and Speda (1938) are interesting in this connection. They were able to demonstrate a decrease in the histamine content of the plasma of cats blood several minutes after the production of artificial hyperventilation for a few minutes. If the hyperventilation is stopped, the plasma regains its previous histamine content after two hours only. The same results were brought about by means of hyperventilation induced by other means such as hyperthermia or cardiazol.

(b) <u>During muscular contraction:</u> - The theory that histamine is responsible in part for the hyperemia which occurs during muscular activity has been expressed by many workers. (Lewis and Grant 1924; Fleisch 1929; Anrep and von Saalfeld 1935).

In 1930 Schulte made alcohol extracts of cat gastrocnemius muscle before and after subjection to maximal tetanus. These were assayed on guinea pig ileum and it was observed that the histamine-like activity of the muscle decreased to two-thirds its original value following stimulation. Similar results have been reported by Alam, Anrep, Barsoum, Talaat and Wininger (1939). They do not state the method of extraction.

Anrep and von Saalfeld (1935) showed that hyperemia in active muscular contraction takes place immediately after relaxation of the muscle, that the hyperemia producing property is quite stable, and that it is not

affected by eserine or atropine. In further experiments Anrep and Barsoum (1935) demonstrated a release of histamine in the venous perfusate from the stimulated gastrocnemius muscle of the dog. Histamine was extracted by the method of Barsoum and Gaddum (1935a). They stated that the increase of the venous blood histamine was due to the activity of the muscle and not simply to nerve stimulation, since no release of histamine occurred if the nerve muscle preparation was first curarized and then stimulated. Anrep and von Saalfeld (1935) observed that curarisation of muscle did not cause the release of any vaso-dilator substances.

However, Alam, Anrep, Barsoum, Talaat and Weininger (1939) found that administration of curare even without stimulation of the muscle is followed by a far greater liberation of histamine than ever observed during contractions of the normal muscle. They do not explain the difference in the results obtained in these experiments as compared to those described above.

(c) Cardiac activity: - Using a heart lung preparation, Anrep, Barsoum and Talaat (1936) investigated the liberation of histamine in the coronary blood during increased cardiac activity. The histamine content of the coronary venous blood was found to be higher than that of arterial blood. If the heart was made to beat against a raised blood pressure or if its activity was increased by the action of adrenaline, an increase in the histamine content of the coronary venous blood was observed, whereas the production of changes in the rate or stroke volume were ineffective. Since the increase of histamine was not found in the arterial blood, it was concluded that the excess histamine must be taken up by the lungs. Other workers however have shown that the histamine content of the lungs is not altered after the administration of adrenaline (MacGregor and Peat 1931; Tarras-Wahlberg 1936). In isolated rabbit hearts, Anrep, Barsoum and Talaat (1936) demonstrated a release of histamine following the administration of adrenaline, but were not able to

show any in the cat heart. Marcou and Parhon (1938) using the method of Barsoum and Gaddum (1935a) extracted arterial and venous coronary blood from a heart lung preparation, before and after raising the blood pressure, and after the administration of ephedrine. An increase in the histamine content of the coronary venous blood was observed when heart activity was increased by raising the blood pressure by the action of ephedrine. They do not state whether their results are expressed as base or as one of the salts.

In an attempt to repeat the work of Anrep, Barsoum and Talaat (1936), Code, Evans and Gregory (1938) were unsuccessful. These workers were unable to demonstrate any increase in the histamine content of the venous perfusate either from heart lung preparations, or from the isolated heart, using the method of Barsoum and Gaddum (1935) and the modification of Code (1937b) for the extraction of the histamine. They criticise the results of Anrep, Barsoum and Talaat (1936) by stating that although they found the cardiac venous blood to have a histamine activity ranging from two to nine times as great as that of arterial blood, in only about 2% of the samples collected did the difference between the arterial and the coronary venous blood lie within the limits of accuracy of the method.

(3) Liberation of Histamine by Cell Stimulation and Injury.

The liberation of histamine from cells by stimulation or injury has been shown in many ways in recent years. Although it was postulated by many that histamine, or a histamine-like substance was released under various conditions such as the anaphylactic reaction in particular, it was not until 1932 that such a demonstration was made by Bartosch, Feldberg and Nagel. Following these experiments, many other workers have shown the release of histamine by many other means, and these will now be considered.

(a) Burns: - In 1927 Harris estimated the histamine content of the skin of the cat. He then compared this with the histamine content of skin which had been burned. If the skin was removed immediately after burning, no change was observed. If however it was allowed to remain intact for some time after the formation of oedema and then removed, the histamine content was greatly reduced.

Bennet and Drury (1931) found that histamine-like substances were released into the perfusion fluid of the isolated cat heart after burning. In perfused hind extremities of cats and rabbits Nagamitu (1935) observed the release of a vaso-dilator substance in the venous perfusate after burning the skin. The vaso-dilator activity was in part destroyed by histaminase. Barsoum and Gaddum (1936) found that an increase in total blood histamine occurred in man after burning of the skin. As a rule the blood histamine equivalent rose to from four to six times its normal value four to six days after the burn. The greatest increase was observed at the time when symptoms of secondary shock became manifest. However no clear evidence of any correlation between the level of histamine in the blood and the clinical condition of the patient was observed. It was thought that other toxic substances might be in part responsible for the condition of shock.

Observations, similar to those of Barsoum and Gaddum (1936) on the increase of the blood histamine following severe burns were made by Code and MacDonald (1937). Other workers however believe that substances other than histamine may play a significant role in the production of the tissue changes and in the resultant symptoms following severe burns. Wilson, Jefferey, Roseburgh and Stewart (1937) found a toxic substance in the bladder contents of rabbits following burns, which did not resemble histamine. Rosenthal (1937a) found a substance in the blood of shoats one hour after they had been subjected

to burns, which was capable of contracting guinea pig uterus, but which lost its activity on being heated to 60°C for three minutes. The substance was not investigated further.

(b) Anaphylaxis: - Lewis (1927) expressed the view that anaphylactic skin reactions were only a special type of cell injury and that the changes observed were due to the liberation of an 'H' substance.

Since the early observations of Manwaring (1911) the liver of the sensitized dog has been thought to play an important role in the release of vaso-dilator substances following the reinjection of the antigen. In 1925 this vaso-dilator substance found in the blood coming from the liver of a sensitized dog, following the injection of an antigen, was found to be similar to histamine in that it was a vaso-dilator substance and contracted smooth muscle. (Manwaring, Hosepian, O'Neill and Moy). The same observations were made by Simon and Brandes (1927).

Later observers were able to demonstrate the release of a substance with similar activity not only in the blood from the liver, but in the lymph flow from this organ in anaphylactic shock (Dragstedt and Gebauer-Fuelnegg 1932). Tinel, Ungar and Zerling (1935) were able to show an increase in gastric secretion following the production of anaphylactic shock in the dog, which they believed to be due to the liberation of histamine.

The release of histamine from the perfused lungs of a sensitized guinea pig after addition of the antigen to the perfusing solution was first demonstrated, and described by Bartosch, Feldberg and Nagel (1932). They identified the substance by various tests, and proved as far as possibe with the exception of chemical isolation, that the active substance was histamine

Their results were confirmed by Daly and Schild (1934) who further noted that the histamine released in the perfusing solution could be inactivated by histaminase. They were able to demonstrate a release of from 0 - 12.8 y of histamine from a pair of guinea pig lungs. Recently however Echague (1938) has been unable to demonstrate an increase in the blood histamine of the sensitized guinea pig following the production of anaphylaxis. He used the method of Barsoum and Gaddum (1935a) and gave the normal amount of blood histamine content in this species as 0.45 - 0.57 y/cc without stating whether it was expressed as base or as a salt.

The release of histamine by the antigen-antibody reaction has been demonstrated independently in vitro by Schild (1937) and Ungar and Parrot (1937). Schild sensitized guinea pigs with ovalbumen, and after waiting about two weeks, he killed the animals, and placed the various tissues in Locke solution after first washing them with Ringer. After remaining in the Locke solution for ten minutes, they were removed and placed in Locke solution to which the antigen had been previously added, and were allowed to remain for ten minutes in contact with this second solution. Both solutions were then tested for histamine. No response was eliciated by the Locke solution which did not contain the antigen, whereas the second gave varying responses depending upon the tissue which had been immersed. Schild obtained the greatest release from the aorta, lungs and uterus, and smaller amounts from the other organs. No histamine was released from the stomach, ileum or liver.

The results of Ungar and Parrot (1937) differ somewhat. They sensitized guinea pigs to horse serum and followed much the same procedure as that outlined by Schild. No activity was found in the solution to which liver, striated muscle or heart had been added. Medium activity was found in that containing spleen, brain or skin whereas the greatest activity was shown by the fluid in which lung, intestine or kidney had been placed.

Code (1938) made a detailed investigation of the blood histamine in dogs during anaphylactic shock, and was able to show that the blood histamine generally reached its maximum within six minutes of the injection of the antigen.

(c) Peptone: - Although both Lewis (1927) and Dale (1929) had postulated that the histamine-like actions of peptone might be due to the release of histamine, due to stimulation of the cells by peptone, the first demonstration of such an action was not made until 1937 by Feldberg and O'Connor. They perfused the isolated lungs of the guinea pig with fluid to which histamine-free peptone was added, and noted a release of 3% of the total histamine content of the lungs. In cats lungs a loss of up to 10% was observed. Dragstedt and Mead (1937) showed that the vaso-depressor substance liberated in the blood of the dog when it is given intravenous injections of peptone is histamine-like in character in that it causes a depression in the blood pressure of the anaesthetized cat under the influence of atropine.

(d) Snake venom: - The similarity between the symptoms produced by the injection of snake venom of histamine, and those of anaphylactic shock has been noted by various observers. (Kellaway 1929; Essex and Markowitz 1930). Kellaway (1929) thought that histamine may be liberated by the cell destruction caused by the action of snake venom. Recently Feldberg and Kellaway (1937a) (1937b) were able to demonstrate the release of histamine from the perfused lungs of the guinea pig and the cat by the action of snake venom introduced into the perfusion fluid. They investigated three different snake venoms, that of the Australian Copperhead (Denisonia Superba), the Indian cobra (Naia Naia) and the North American rattlesnake (Crotalus Atrox). Results from the use of these three venoms varied only quantitatively. Allthree caused broncho-constriction, swelling of the lungs due to accumulation of

fluid and the appearance of glassy patches. That the histamine assayed in the perfusates was part of the original histamine content of the lungs, and not histamine produced by the action of the venom itself, was shown as follows: The histamine content of different sections of the left and right lungs was estimated and the values were shown to be within 10%. They made use of this as a control. This was done by first removing one lobe and estimating its histamine content. The rest of the lung tissue was then perfused with various substances, and at the end of the experiment, it was extracted for histamine. The difference in the amounts of the two sides indicated the amount of histamine released from the perfused lung.

In unpublished results Feldberg and Kellaway (1937c) were able to show that cobra snake venom will cause the release of histamine from dogs liver. They also showed that there is a parallelism between the original histamine content of the lungs and the effect of the venom. Young kittens, in whom the histamine content of the lungs is low show less symptoms from snake venom than do old cats in whom the histamine content of the lung is high. Feldberg and Kellaway (1937b)..However the amount of cell injury varied in this same way, that is greater cell injury and destruction occurred in the lungs of older cats, than in young kittens. Dragstedt, Mead and Eyer (1937) studied the effect of intravenously injected crotalin (Crotalus Atrox) in the anaesthetized dog. They confirmed the finding of Feldberg and Kellaway (1937a) that histamine may be liberated by the action of crotalin, but came to the conclusion that the amount of liberated histamine is inadequate to account for the degree of vascular reaction produced.

(e) Bee venom: Feldberg and Kellaway (1937d) demonstrated that bee venom had the power of liberating histamine from the perfused lungs of the guinea pig when it was added to the perfusion fluid. They used a solution of bee venom

containing the equivalent of 50 stings per cc. The rate of release of histamine increased in the first 60 - 90 minutes after the injection and then decreased. They later (1937e) showed that histamine is liberated from the perfused lungs and liver of the dog.

(f) Staphylococcus toxin: - Bacterial poisons were also considered by Lewis (1927) to act in part by a liberation of 'H' substance due to cell injury. Feldberg and Keogh (1937) were able to show that histamine could be liberated from the perfused lungs of the guinea pig and cat, by the action of staphylococcus toxin. They chose this particular bacterial toxin because it is known to have a much shorter latent period in the production of symptoms than most others (Kellaway, Burnett and Williams 1930). A latent period of about 10-40 minutes after the administration of the toxin was followed by an output of histamine from the perfused lungs. With the doses of toxin used, the histamine output amounted to between 4 and 15% of the original lung histamine.

In later observations Feldberg, Kellaway and Keogh (1937) noted that increasing the toxicity of the staphylococcus toxin did not cause a greater release of histamine. They explain this by the fact that the toxin injures certain cells only, in particular those of the peribronchial district, and once the histamine is liberated from them, no other cells are left upon which the toxin may act.

(g) Chemicals: - Bartosch (1936) perfused the isolated lungs of the guinea pig, and allowed various substances to enter the tracheal cannula. A dose of 0.02 cc of heptan, octan, benzol, toluol or petroleum ether caused fixation of the lungs and the release of histamine in the perfusion fluid. The first three substances were most active in causing a release of the amine. Ether, chloroform and chlorethyl did not have any effect.

Analagous results were found by perfusion of the rabbit ear with these substances.

Similar results were obtained by Garan (1938b) who demonstrated a release of histamine from the isolated perfused lungs of the guinea pig by the inhalation of ammonia, acrolein chlorpilarin and toluol.

Heubner and Bachman (1937) found that perfusion of the intestine of the etherized cat with $HgCl_2$ caused a liberation of histamine in concentration of 0.02 - 0.1 mg%. Arsenic and sodium cyanide did not possess this property. Similar experiments on the rabbit however were negative.

(h) Trypsin: - Recently the effect of trypsin has been studied by Roche E Silva (1938). He found that the addition of trypsin to the perfusing fluid of an isolated guinea pig lung preparation would cause a release of histamine in the venous perfusate. He employed the method of Code (1937b) for extraction.

(i) Curare: - That curare had a peculiar effect on the isolated perfused gastrocnemius muscle preparation of the dog was noted by Anrep and Barsoum (1935a). Alam et al (1939) as has been mentioned previously, have made the observation that the administration of paralysing doses of curare to such a preparation causes the release of large amounts of histamine. The nature of the mechanism does not depend upon the paralysing action of curare on the motor nerve endings since the effect is observed also after degeneration of the muscle nerve. They concluded that the histamine which appears in the venous perfusate by the action of curare on muscles, is not formed from some precursor substance in the muscle by curare, but is liberated from the muscle tissue where it must exist in loose combination. This was shown by the fact that extraction of the muscle before and after curare could account for the amount of histamine liberated in that the histamine content of muscle was always diminished by an amount equal to that liberated in the venous perfusate.

(j) Ultraviolet light: - Following the exposure of human skin to sunlight, or to ultraviolet rays of a wave length of about 300 m. u., a vaso-dilatation of the blood vessels of the skin and erythema occurs, usually within an hour of the exposure. The changes resemble those observed in the 'triple reaction' as described by Lewis, and according to him are due to a local release of the 'H' substance. This response is the general reaction of the skin to stimulation or injury and varies only in the latent period of onset with different types of stimuli.

Ellinger (1928) observed that histamine could be produced from histidine by the action of ultraviolet light of a wave length of 250 m. u. Only about one five-hundredths of the histidine was decarboxylated. Ellinger believed that this may be the sequence of events in human skin following irradiation, namely that histamine might be derived from histidine rather than existing in the tissue as such in loose combination. However he was not able to demonstrate an increase in the histamine of the skin after exposing various areas of the body to ultraviolet light, using guinea pigs and swine.

Bourdillon, Gaddum and Jenkins (1930) repeated Ellinger's (1928) work and found that whereas ultraviolet light of a wave length of under 265 m. u. would produce histamine from histidine, such light could not penetrate human skin, and was not found in sunlight. They also noted that light of a wave length of 300 m. u. which is found in the atmosphere and will cause erythema of skin has not the capacity to produce histamine from histidine in vitro. On these grounds Ellinger's theory is rejected by Gaddum and Dale (1936).

Other workers (Kawazuchi 1930; and Isobe 1936) have observed an increase in the histamine content of the skin of guinea pigs and rabbits following irradiation with ultraviolet light. Tarras-Wahlberg (1937) has recently studied the effect of ultraviolet irradiation of the skin of rabbits and cats. He does not state what wave length was used. Using one side of

the body as control, he exposed areas on the opposite side for varying lengths of time. The skin was removed at times anywhere from five minutes to four days after the exposure. It was then extracted by the method of Barsoum and Gaddum (1935a). He noted an increase in the weight of the skin of both species due to oedema followed by a decrease in weight. In the rabbit, a rise in the histamine content of the skin was noted, occurring after five minutes and then reaching its maximum in 24 hours, followed by a progressive decrease to zero in 48 hours. In the cat however, the histamine content of the skin began to decrease immediately and fell progressively to zero by the third day. Tarras-Wahlberg explains these findings on the 'skin-blood-histamine' quota. Rabbit skin contains 14.7 y per gram, and the blood histamine is 7.3 y per cc according to him. This gives a ratio of two. In the cat however, the skin contains 30.8 y histamine per gram, and the blood has a histamine content of 0.015 y per cc giving a ratio of 2000. Thus increased blood flow and transudation following irradiation carries much more histamine to an area in the rabbit than it does in the cat. He believes that this explains the differences previously reported. It should however be noted that conflicting results have been given for the same animal.

(k) Schwartzman reaction: - If a rabbit receives an intradermal injection of a bacterial filtrate which is followed twenty-four hours later by an intravenous injection of the same or another filtrate, a localized reaction at the site of the first injection will occur within one half to one hour following the second or intravenous injection. This reaction is usually of an acute haemorrhagic variety. It will occur in any locality in the animal, providing that a primary injection has been made. This is known as the Schwartzman phenomenon. (Schwartzman 1936).

Rocha E Silva and Bier (1938) estimated the histamine content of rabbit skin, using the method of Code (1937b) in normal rabbits and in rabbits in which the Schwartzman phenomenon had been produced. Histamine content of normal rabbit skin was found to vary from 4.27 to 12.7 y per gm, with an average of 7.0 y per gm. (They do not state whether or not this is expressed as base).

Fragments of skin removed from haemorrhagic sites immediately following production of the Schwartzman phenomenon varied from 12.5 to 56.0 y per gm, and a decrease was noted in one specimen taken 24 hours after the second injection, however the skin taken from uninjected areas showed higher values than normal, one being 25.8 y/gm.

They believe that histamine is liberated during this phenomenon therefore and acts as an adjuvant by enhancing fragility and permeability, thereby producing haemorrhagic areas.

(1) Inflammation: - ~~Because of~~ USING various irritants, Loos (1935) produced inflammatory changes in rabbits ears. The histamine content of the ears was determined by a method of extraction in which the tissue was treated with alcohol and the proteins precipitated with phosphotungstic acid. Final assay was carried out on the guinea pig ileum. A 40% increase of the histamine content of ears in which inflammatory changes had been produced was observed in some cases.

Riesser (1937) found that the histamine content of the skin of the rabbit increased from four to five times the normal amount after the production of inflammatory changes caused by the injection of an irritating substance such as mustard oil.

Recently Bier and Rocha E Silva (1938b) have shown that the injection of extracts made from the skin of the rabbit would produce the same result as histamine, if injected subcutaneously in the rabbit. They infer that these

extracts contain histamine, and that this histamine is responsible for part of the inflammatory changes, namely increased permeability of the capillaries and vaso-dilatation.

(m) Trauma: - Minard (1937b) using an electrodialysis method for the extraction of histamine from blood observed an 80% increase of the histamine content in the venous blood from the traumatized limb of the dog. No change from the normal was seen either in arterial or venous blood before trauma was inflicted or after the animal was in profound shock. Holt and MacDonald (1936) however, as well as MacDonald and Woolfe (1938) were unable to demonstrate any constant or significant increase in the histamine content of the venous blood from traumatized limbs of cats, as compared with that obtained from normal limbs. They took their specimens usually just before the death of the animal however. Both of these workers used the method of Barsoum and Gaddum (1935a).

(n) Intestinal strangulation: Aird and Henderson (1937) demonstrated a release of from 0.5 to 4.0 mg of histamine (base) in the exudate surrounding a loop of strangulated bowel in cats. The exudate was collected by surrounding the isolated bowel with a rubber bag. They used the method of Barsoum and Gaddum (1935a) for extraction of the exudate.

Using the method of Yokoyama (1936) Akiyama (1937) extracted the tissues of the dog in which he had produced intestinal obstruction by removal of a small piece of bowel, and ligature of the blind ends. An increase of five times the normal histamine content was observed in the lung and liver, whereas the kidney histamine content was increased 16.5 times the normal.

Maycock (1938) using the method of Barsoum and Gaddum (1935a) has shown that histamine may be liberated in the peritoneal transudate surrounding short non-viable loops of intestine and that the total depressor action of such transudates may be accounted for by histamine and choline.

(o) Insulin shock: - It has recently been shown (Bartelheimer and Afendulis 1938) that histamine may be liberated into the blood of the guinea pig following the production of insulin shock. The increase is observed at the height of the shock. Insulin alone is ineffective if shock-producing doses are inhibited by the administration of glucose. Extraction of the blood was carried out by the method of Barsoum and Gaddum (1935a) and assayed by the cats blood pressure method.

(p) Portal obstruction: Gulzow and Afendulis (1938) produced portal obstruction in dogs by partial to complete ligature of the portal vein. Complete obstruction causes death within two to five hours. Lesser degrees of obstruction produces a shock-like condition, concomitant with the appearance of a severe gastritis and of a marked increase in the portal and systemic blood histamine as assayed on the blood pressure of the anaesthetized cat under the influence of atropine.

"The Role of Histamine in Various Pathological Conditions.

Recent as well as earlier observations have indicated that histamine may play a major role in the production of various symptom complexes such as the anaphylactic reaction and in the production of shock-like conditions. These will now be discussed.

A. Anaphylactic Shock:

Discussion of the earlier theories of anaphylactic shock will not be entered into here, suffice it to say that they have for the most part been discarded. This subject is of interest in this discussion mainly because of the fact that the majority of the symptoms observed in anaphylactic shock have a marked similarity to the symptom complex which follows upon an intravenous injection of histamine in the same species of animal.

Portier and Richet (1902) made the first observations on the sensitizing of an animal to a naturally occurring protein. This was produced in the dog by the parenteral administration of a protein in sublethal doses, to which the animal became sensitive. Theobald Smith (1906) then showed that a guinea pig might become very sensitive to an injection of horse serum if a small dose of this harmless substance had been previously injected. This was confirmed by other workers, and it was established that the condition of hypersensitivity was dependent upon the production of antibodies.

Weil (1913) showed however that the condition of anaphylaxis brought about by the artificial production of antibodies, was increased as these antibodies disappeared from the blood and reached its peak when the antibodies completely disappeared from the blood stream.

In the meanwhile the observations of various workers pointed towards a marked similarity between the symptoms of anaphylaxis and peptone shock (Biedl and Kraus 1910) and those produced by an intravenous injection of histamine (Dale and Laidlaw 1910). It was noted that in the guinea pig an acute emphysema occurred in anaphylactic shock due to extreme spastic bronchiolar constriction. In the dog on the other hand there occurred marked congestion of the liver and of the portal circulation, accompanied by collapse of the general circulation due to peripheral vascular dilatation. Certain differences were also apparent. The most outstanding one being that, whereas the coagulability of the blood was little affected by histamine, peptone usually caused a slight retardation in coagulation time and marked prolongation of the coagulation time was always noted during anaphylactic shock.

In the rabbit, the resemblance between histamine shock and anaphylactic shock was established by the work of Coca (1914) who showed that constriction of the pulmonary blood vessels with failure of the right heart occurred in both.

The great difficulty encountered in the attempt to produce anaphylactic shock in mice and rats is paralleled by their marked resistance to histamine. It is also notable that following adrenalectomy the resistance of these animals to histamine decreases markedly and anaphylactic shock may be produced (Wyman 1929; Suden 1937).

In (1911) Manwaring had observed that anaphylactic shock in the dog was associated with the appearance of a substance in the blood which resembled histamine in its action. He later showed that anaphylactic shock could not be produced in dogs and guinea pigs, in which the liver had been previously removed. Since removal of the liver did not affect canine histamine

shock according to Manwaring (1922) and his associates, he concluded that canine histamine peptone and anaphylactic shock were not identical physiological reactions. In a recent publication, however, it has been noted that anaphylactic shock may be produced in dogs in which the liver has previously been removed (Waters, Markowitz and Jaques 1938).

It was not until 1927 that Lewis proposed the theory of the liberation of histamine on the basis of a release of this substance from cells by means of stimulation or by injury of some sort. He devised many types of experiments to show that the 'H' substance as he termed it, could be released from cells by stimulation, and in his laboratory, Harris (1927) showed that following severe scalding the histamine content of the skin of the cat was diminished. Although no experiments had as yet been carried out to show that the anaphylactic reaction may release histamine by cell injury, Lewis included this in his theory. This was emphasized by Dale (1929) who postulated that intracellular reaction between antigen and antibody causes cellular injury which liberates histamine and other substances.

On such a basis then it became easier to understand why the injection of an antigen into a sensitized guinea pig produces death from suffocation, since the release of histamine which would be presumed to occur would act on the sensitive bronchial musculature in the same way as did an intravenous injection of histamine. And in the dog, the liver becomes engorged with blood due to narrowing of the hepatic veins, which are constricted by the action of released histamine.

Direct evidence for the proof of this theory was lacking although Manwaring (1911) had stated that the blood of a dog in anaphylactic shock had histamine-like qualities. In 1932 Gebauer-Fuellneg, Dragstedt and Mullenix noted that the thoracic duct lymph of a dog in anaphylactic shock contained a substance which because of some of its physiological and chemical properties

must be histamine.

The first completely worked out evidence in support of the histamine theory in the guinea pig however was produced by Bartosch, Feldberg and Nagel (1932). These workers demonstrated the release of histamine in the venous perfusate of the isolated lungs of the sensitized guinea pig following introduction of the antigen. This and subsequent observations (Daly and Schild 1934; Schild 1937; Ungar and Parrot 1937 etc.) have already been described in a previous section.

It was further shown by Schild (1936) that the quantity of histamine released by the lungs varied with the degree of sensitisation and is proportional to the response of the vascular and bronchial musculature. In order to establish whether contraction of smooth muscle 'per se' could cause the release of histamine, he added barium chloride to the perfusing solution. This caused marked smooth muscle contraction with resulting broncho-constriction. No release of histamine however was observed.

Since certain substances have been shown to cause anaphylactoid reactions (Hanzlik and Karsner 1920) in the guinea pig, these were also investigated. Addition of kaolin, agar, KCl and $CaCl_2$ produced an anaphylactoid response of the guinea pig lungs, but in no case was histamine released.

Schild concluded therefore that smooth muscle contraction does not release histamine, and that the liberation of histamine in anaphylaxis is responsible for the effect on smooth muscle.

The question as to whether sensitisation of an animal by means of subcutaneous injection of a foreign protein might alter the histamine content of tissues was investigated by Watanabe (1930), who was able to show an increase in the lung histamine of the sensitized guinea pig, as compared with that of the normal animal. The average histamine content for normal lung tissue was given by him as being 22 y/gm, whereas that obtained after

sensitisation was found to be 80 y/gm. He further showed that following the production of anaphylactic shock in the same animal, there was a decrease of the lung histamine content to 3 y/gm in 24 hours. The histamine content of the lung tissue was determined by making alcoholic extracts and assaying these upon the blood pressure of the atropinized anaesthetized cat.

However no other workers have been able to confirm these results using newer methods. (Bartosch, Feldberg and Nagel 1932; Daly, Peat and Schild 1935).

The recent work of Code (1938) has demonstrated clearly that the production of symptoms of anaphylaxis in the dog, that is the marked drop in blood pressure etc. are coincident with the release of histamine into the blood plasma. This is of great significance, for it is known that large intravenous doses of histamine may be tolerated by the dog when injected slowly. It would seem therefore that the symptoms are dependent upon the rapid release of histamine into the blood plasma, where it is free to exert its action.

Although it is now accepted that cell injury which occurs by the intracellular reaction of an antigen with the antibody causes a release of histamine, other substances may also be released. For instance, the prolongation of blood coagulation time, especially prominent in the dog during anaphylactic shock does not occur by the action of histamine itself. This change in the blood coagulability has been explained by the release of antithrombin-like substances (Nolf 1917). It was believed that the antithrombin-like substance was released from the liver and this has been verified by the work of Waters, Markowitz and Jaques (1938). They first produced anaphylaxis in normal dogs, and estimated the heparin released in the blood, confirming the findings of Eagle, Johnston and Ravdin (1937), and of Quick (1936) for peptone shock. There is a marked release of heparin within the first ten

minutes after injection of the antigen into an etherized sensitized dog, or after twenty minutes following the injection of peptone into a normal dog. Another dog was previously sensitized to horse serum, to which 1% alum had been added. Six weeks after, the liver of this animal was removed and the antigen given intravenously (20 cc normal horse serum). There was a marked reaction typical of anaphylaxis with fall of blood pressure. In this animal however, no heparin was liberated, indicating that its source MUST BE THE LIVER in the anaphylactic reaction. ~~has raised the question as to whether or not histamine may be liberated from this organ by certain workers.~~

Wachstein (1933) added antigen to isolated strips of guinea pig and rabbit heart muscle from normal and sensitized animals. He found no difference in the response and came to the conclusion that the increase in the rapidity of the heart beat was due to the liberation of histamine from the lungs in anaphylactic shock.

Went and Lissak (1935) were able to demonstrate that choline was liberated from the heart during anaphylaxis and that this was responsible for the changes occurring in this organ during anaphylactic shock. They were able to inhibit the production of shock in the isolated sensitized heart after administration of the antigen by atropinisation of the organ. It was further shown by Lissak and Kokas (1935) that the production of a drop in blood pressure, bronchosparm and contraction of the spleen, occurring during anaphylactic shock in the dog, could be reproduced by the action of choline injected intravenously. They believe therefore that the symptoms of anaphylaxis in the heart are due to the action of choline.

Recently however Cowles and Andrus (1938) have noted that the effect of histamine or of the anaphylactic reaction are identical in the isolated heart of the guinea pig. They made the interesting observation that although the rate and amplitude of the heart beat were increased, there was

marked reduction in the rate of flow through the coronary vessels. It was noted that whereas atropine did not affect the rate or amplitude of the heart beat, the constriction of the coronary vessels was suppressed in both cases.

The observations of Went (1938) and of Cowles and Andrus (1938) explain the inhibiting action of atropine in anaphylactic shock, in the guinea pig, but the opinion of Went (1938) that the response of the heart to anaphylactic shock is due to the liberation of choline-like substances is not in accordance with the findings of Cowles and Andrus (1938), for Went and Lissak (1935) state that the response of the heart of the sensitized guinea pig to the antigen is characterized by a definite arrythmia followed by heart block and finally a return to normal. Cowles and Andrus (1938) on the other hand observed an increase in both the rate and amplitude. There was also some arrythmia. Ratnoff (1939) produced varying degrees of anaphylactic shock in rabbits. He was unable to demonstrate any release of acetyl-choline, although the shock was severe enough to cause death of the animal.

The role of the sympathetic nervous system in both anaphylactic and histamine shock has been studied by Lissak and Hodes (1938). Removal of the sympathetic nervous system does not affect either histamine or anaphylactic shock according to these workers. The drug 933 F (piperidinomethyl-benzodioxane) inhibits the action of anaphylactic shock but does not alter the effect of histamine. Ergotoxine has no effect on either type of shock. These studies were carried out on cats.

The production of anaphylactic shock has been observed following the administration of antigen to sensitized monkeys after decerebration carried out just before the administration of the antigen. (Davidoff, Kopeloff and Kopeloff 1937).

B. Peptone Shock:

As early as 1880 it was known that intravenous injections of peptone produced profound shock in animals (Schmidt-Mühlheim). Biedl and Kraus (1910) showed the marked similarity of this type of shock to that produced by histamine and the anaphylactic reaction. Later when histamine was found to be a constituent of peptone (Abel and Kubota), it was thought that this histamine was responsible for the production of the symptoms, even though it was present in very small amounts. This was dispelled by the work of Hanke and Koessler (1920) who produced peptone shock with histamine free peptones. It was further observed that the injections of peptone into dogs lead to a rapid desensitisation of this animal to peptone. (Biedl and Kraus 1910; Abel and Geiling 1924). The incoagulability of the blood produced in peptone shock has also been observed by many workers (Biedl and Kraus etc.).

In 1937 workers from two different laboratories, Dragstedt and Mead in the United States, and Feldberg and O'Connor in Australia demonstrated the release of histamine by the action of peptone. Dragstedt and Mead using dogs, observed the liberation of histamine into the blood and lymph following the intravenous injection of peptone. Feldberg and O'Connor observed the same phenomenon in the isolated perfused lungs of the guinea pig and cat. According to these latter workers, the cell injury produced by peptone in the lung resembles that caused by the anaphylactic reaction and the amounts of histamine liberated are of the same order.

The incoagulability of the blood produced by peptone is regarded as a direct action of this substance, since it is not produced by histamine. The work of Waters, Markowitz and Jaques (1938) has shown that it is due to the release of heparin from the liver by the action of peptone.

The desensitizing action of repeated doses of peptone has been studied in relationship to anaphylactic shock in the dog by Mead, Dragstedt and Eyer (1937)

They have shown that it cannot be due to a depletion of the histamine stores in the tissues of the body, since desensitizing against peptone by repeated doses of this substance protects the animal against the action of peptone, but will not protect against the production of anaphylactic shock in a sensitized animal. That is histamine is still present in sufficient quantities to produce anaphylactic shock, even though the animal might be refractory to peptone. This also diminishes the possibility that the production of peptone may be an intermediate step in the anaphylactic reaction, since if this were so, desensitization against peptone would also protect against anaphylaxis.

C. Histamine in Relation to Allergy:

Although it is now definitely established that histamine is one of the basic factors responsible for the production of the anaphylactic symptom complex, the evidence is not as convincing with regard to its relationship to allergic manifestations in man. It is convenient to divide the various conditions into two large groups, those on the one hand which are supposedly due to the sensitisation by foreign proteins or chemicals and those which are due to changes in physical environment.

On the basis of what has been written in the previous sections however it is assumed that such manifestations are due to the liberation of histamine or histamine-like substances from the cells of various parts of the body.

It is convenient to assume that since all of these manifestations are due to the release of histamine by cell injury or cell stimulation, then the type and location of allergic manifestations will depend upon which cells have become sensitized. In the case of asthma, one supposes that certain cells of the lung are sensitive, in mucous colitis, those of the intestine,

in urticaria, those of the skin etc. Evidence for this has been provided by the following workers.

Kalk (1929) demonstrated a secretion of gastric juice in patients with marked dermatographia. Production of wheals by scratching was followed by a marked increase in the acidity of the gastric juice, due presumably to the liberation of histamine into the blood. Normal individuals gave no response. Grant, Pearson and Comeau (1936) have described the production of urticaria, erythema and wheals in patients responding to heat, exercise and emotion.

The occurrence of achlorhydria in patients with asthma, mucous colitis and chronic skin disease has frequently been noted (Voss and Voss 1937) (Hurst 1936). That the achlorhydria in these conditions is probably due to the chronic liberation of histamine has been demonstrated by Voss (1938) who produced achlorhydria in rats by the chronic administration of histamine.

With the recent development in technique for the determination of histamine in the blood, investigation of the blood histamine in man during asthmatic attacks, urticaria, migraine and serum sickness has been done. These will now be considered.

(1) Urticaria: - Cerqua (1936) using the method of Barsoum and Gaddum (1935) obtained an increase in the blood histamine in six cases of urticaria in acute attacks. His figures are 0.18 - 0.3 y/cc. (normals 0.05 - 0.06 y/cc). After six hours the blood histamine returned to 0.08 - 0.10 y/cc.

(2) Serum sickness: - Cerqua (1936) was unable to demonstrate any increase in the blood histamine during the crisis of serum sickness. Parrot (1938) on the other hand noted a slight rise (0.17 y/cc) in three patients.

(3) Asthma: - Code and MacDonald (1937) were unable to demonstrate a rise in the histamine content of the blood of asthmatics, nor in 18 cases of chronic bronchitis. Similar findings were reported by Riesser (1937). Cerqua (1936) on the other hand was able to demonstrate an increase. He obtained figures as high as 0.25 y - 0.3 y/cc in patients during asthmatic crises. Normal or slightly raised values were found between attacks. Parrot (1938) noted a moderate rise in two cases.

The possibility that acetyl-choline might be a factor in the production of asthma led Milhorat (1938) to study the choline esterase content of the blood of such patients. In the five which he examined great variation was noted, indicating that no definite increase or decrease of acetyl-choline was present in the blood of such patients.
In 1930 Knott and Oriell demonstrated the presence of a histamine-like substance in the sputum of patients suffering with asthma. They used simple saline extraction. Using a more reliable method, Riesser (1937) was able to confirm this finding.

(4) Migraine:- Parrot (1938) examined the blood of patients suffering from migraine, and noted rises in the histamine content of the blood occurring within fifteen minutes after the onset of the attack. He also observed that a histamine-like substance appeared in the urine.

As with cases of asthma, Parrot (1938) was able to precipitate typical attacks in patients suffering from migraine by the injection of histamine subcutaneously. He notes however that in the actual migrainous attack, and in that provoked by an injection of histamine, the symptoms are coincident with a rise in the blood pressure which he believes is the cause of the symptoms. The rise in blood pressure is perhaps due to the action of histamine on the adrenals in which a release of adrenaline occurs as is known to occur (Szczygielski 1932).

(5) Physical Allergy: - Hypersensitiveness to cold has been studied by Horton, Brown and Roth (1936) and a review of the subject was presented by Goodson (1938). It differs from the type of allergy first discussed in that the allergic manifestations are apparently brought about by physical changes, i. e. collapse due to exposure to cold, urticaria due to overheating or sunlight.

In such patients there is a sensitivity to certain physical agents which is inherited in many cases. Just what determines this sensitivity is unknown, however the production of the symptoms, i. e. fall in blood pressure, urticaria etc. may well be due to the release of histamine from the stimulated or injured cells, just as the release of histamine by antibody-antigen reaction causes histamine to be released in cases of allergy.

The recent work of Karady (1939) is interesting in this respect. He took guinea pig serum and exposed part of it to heat and another part to cold without causing precipitation of the proteins. The serum was then injected into guinea pigs. After a period of incubation, he again reinjected similarly treated serum into the sensitized animals. It was noted that anaphylaxis was produced by the serum exposed to cold in those animals sensitized with such serum, whereas the giving of a provocative dose of serum exposed to cold produced no symptoms in animals sensitized to heated serum.

Going one step further, one group of guinea pigs was treated by immersing the hind limbs into ice water for a few minutes. A second group was treated by immersing the hind limbs into hot water ($60^{o}C$). After an interval of two weeks, both groups were exposed to the same treatment. Anaphylactic symptoms appeared only in those animals which were exposed to the same treatment which they had received previously, i. e. re-exposure to cold produces the symptoms of anaphylaxis, whereas exposure to cold followed

in two weeks by exposure to heat produced no symptoms.

Karady (1939) believes that the extreme heat or cold causes changes in the proteins so that they may then act as antigens and refers to these as 'endoantigens'.

Recently however evidence has been presented which does not support the view that histamine is responsible for the production of the allergic wheal.

Abramson and Alley (1937) developed an iontopheric method which permits the detection of histamine in dilutions as high as 1 : 5 000 000 by the formation of wheals in the human skin. When histamine is administered by the galvanic current so that a wheal forms in the human skin, the positive pole is applied to force the positively charged histamine ions into the skin, with the subsequent production of a histamine wheal. Abramson, Engel, Lubkin and Ochs (1938) further found that if the negative pole be applied to the surface of a histamine wheal (formed previously by either direct introduction of histamine as in the scratch test, or by iontophorosis as described above) sufficient histamine is transported out of the wheal by this reversed iontophoresis to form secondary wheals in new areas of the skin.

They then produced wheals in sensitive patients using (1) ragweed, (2) timothy; (3) ultraviolet light and (4) a case of severe dermatographism in whom the wheals were produced by stroking. If histamine were present it would then be possible to remove it by placing a negatively charged pole on the wheal (reversed iontophoresis). In no instances however was histamine obtained.

A good review of the recent work on allergy has recently been presented by Rackemann (1939).

Use of Histamine, Cortin and Vitamin C as Therapeutic Agents in Allergic Manifestations: - The results reported by various investigators on the effects of pretreatment with histamine, or the use of cortin or of vitamin C in allergic manifestations have on the whole been unsatisfactory.

Dzsinich (1935) found that daily doses of histamine given subcutaneously to patients with urticaria and bronchial asthma resulted in lasting improvement with relief from symptoms. This has not however been confirmed by other workers.

The use of cortin in the treatment of asthma and allied conditions has been studied by Pottenger, Pottenger and Pottenger (1935) who reported favourable results in the treatment of 30 cases from a group of 50 patients with asthma. The report is concerned only with results observed within the first two weeks of treatment.

Wilmer and Miller (1936) treated a group of 72 asthmatic patients with suprarenal cortical hormone. This was injected intramuscularly in a dosage of 1-4 cc for seven days until a total of 20-80 cc had been given. Complete relief was obtained by 13%, partial relief by 29% and no relief by 57%.

Recently Prickman and Koelsche (1938) treated 19 patients of whom 16 had asthma and the others had various allergic manifestations. They gave sodium chloride by mouth and cortin intravenously. No specific benefit was observed.

Recently Keeney, Pierce and Gay (1939) have prepared a new therapeutic agent by making an emulsion of epinephrine in vegetable oil, 1 cc of which contains 2 mg of epinephrine. Instead of the usual fleeting response obtained by an injection of epinephrine, they have been able to prolong the effect for 8-9 hours. This is of considerable value in the treatment of acute asthma and urticaria, relief being obtained from a single injection which may last from 8-16 hours.

Treatment of cases of asthma with vitamin C has been unsatisfactory (Epstein 1936; Hunt 1938).

D. Relation to Inflammation:

In his extensive work Lewis (1927) postulated that the changes which occur following injury, or stimulation to the cells of the skin, namely the 'triple response' may also occur during the process of inflammation.

According to Loos (1938) histamine will reproduce all of the inflammatory changes observed in the skin of man, with the exception of inflammatory leucocytosis. He showed however that histamine will increase the phagocytic property of horse leucocytes in vitro.

It has been noted that inflammatory areas are capable of retaining pigments (Menkin 1936; Loos 1938; Bier and Rocha E Silva 1938). If a dye has been injected into an individual who has urticaria, or wheals produced by the intracutaneous injection of histamine, the dye will accumulate in the fluid of the wheal in both cases. Loos (1938) is of the opinion that this accumulation of the injected dye is caused first by the increased permeability of the capillaries, produced by the histamine, and secondly by the increased phagocytic property of the white blood cells.

On the basis of a study of inflammation exudates, Menkin (1938a) (1939) has stated that histamine, although present in the inflammatory exudate cannot adequately account for the primary mechanism of increased capillary permeability. That it will not cause polymorphonuclear leucocytic infiltration has been stated by Loos (1938). Henlein's results (1935) are not in agreement with this.

Menkin (1938b) has succeeded in isolating a crystalline nitrogenous substance from such exudates which is extremely active in inducing prompt increased capillary permeability. Further it has chemotactic properties which may be demonstrated either in vitro or in vivo. Within 15-30 minutes following its injection there is an accumulation of leucocytes about the lumen of small vessels which first adhere and soon migrate into the extracapillary spaces.

Menkin has proposed the name of 'leukotaxine' for this substance. The purified substance does not resemble histamine in any way. Bier and Rocha E Silva (1938) have criticised the work of Menkin. They confirmed the finding that histamine increases the permeability of the capillaries as shown by the accumulation of trypan-blue from the circulating blood in areas injected with histamine, or with skin extracts, or inflammatory exudates. In a later paper Rocha E Silva and Bier (1938) claimed that alkaline hydrolysis which inactivates histamine when in combination with tissues, also inactivates the permeability factor of the inflammatory exudate. On these facts, and on a comparison of some of the properties of histamine and leukotaxine as given by Menkin, they are of the opinion that leukotaxine and histamine are one and the same substance.

Menkin (1939) on the other hand has extracted purified samples of leukotxaine for their histamine content and was unable to demonstrate any. He also has shown that leukotaxine is inactive when tested on the guinea pig ileum, the cat blood pressure and that furthermore alkaline hydrolysis fails to dissociate the chemotactic from the permeability factor.

In 1935 Eppinger, Kaunitz and Popper published a monograph called 'Die seröse Entzündung', dealing with morphological changes occurring in various tissues of the organism. The underlying pathological process was described as an increase permeability of capillaries. They believed histamine to be a causative factor. Rössle (1935) expressed the same opinion. Eppinger and Rössle (1935) state that serous inflammation is characterized by the passage of plasma proteins through damaged capillaries into the tissue spaces and is accompanied by oedema.

Henlein (1935) administered histamine intravenously to rabbits over a period of one month and was able to reproduce changes characterized by degeneration of the parenchyma of organs, consisting of separation of the endothelial lining of small vessels, localised swelling of the vessel wall

which may be of a granulamatous nature. He also observed plasma exudation through the vessel wall to the surrounding connective tissue, leucocytic migration and increase in eosinophiles. Granulamatous formation occurred around the vessels, and thrombi were produced in the smaller vessels. The changes occurred mostly in the heart, lung and liver. Such changes, according to Henlein, greatly resemble those observed in animals dying of anaphylactic shock and in 'hyperergic inflammation'.

Investigation into histamine intoxication was also carried out by Eppinger (1936). He was able to produce morphological changes by means of chronic histamine administration. He was however unable to show an increase in the histamine content of the tissues following the production of serous inflammation by means of irritants. Repeating these experiments Kaunitz, Neugebauer and Schweiger (1938) found little change in the histamine content of the liver and kidney of the guinea pig. There was if anything a tendency to decrease. Riesser (1937) stated that the skin of rabbits which had been subjected to the action of mustard oil in order to produce inflammation, contained more histamine than did normal skin. It is also interesting to note that Riesser (1937) was able to demonstrate the presence of histamine in the sputum of patients with tuberculosis and chronic bronchitis as well as in that of patients with asthma.

E. Histamine and Gastric Ulcers:

The production of stomach ulcers by the chronic administration of histamine subcutaneously, has been described in rats (Buchner, Siebert and Molloy 1929). These animals received injections every second day, and were starved before each injection. McIlroy (1928) described the production of ulcers in cats. Harde (1932) noted similar results in mice and guinea pigs. In all of these investigations the production of ulcers was possible only if the animals were starved. Brummelkamp (1933) states that gastric ulcers may

also be produced in rats by starvation alone. He gave food for one day, then starved the animals for two, allowing them to have only water.

O'Shaugnessy (1931) injected histamine directly into the muscular coat of the stomach of the cat and produced ulceration.

Henlein and Kastrup (1938) gave daily intravenous injections of histamine to cats, starting with a small dose and increasing until the last dose was from 1.5 mg to 8.1 mg. These were continued for one month. A regular increase in the free and combined acid of the stomach was observed. However neither ulcers or erosions were found in the gastric mucosa but marked oedema and small haemorrhages were noted.

They are of the opinion that the production of such changes in the mucosa is due to the toxic action of histamine itself, and that the increase in hydrochloric acid is secondary.

Voss (1938) on the other hand produced achlorhydria in rats by the chronic administration of histamine subcutaneously; 6.0 mg of base were given daily for from 6 to 44 days. He did not describe the presence of lesions in the stomach.

F. Traumatic Shock:

Traumatic shock, because of its high incidence during the Great War stimulated the interest of many workers in an effort to determination the mechanism of its causation. Since that time, a great deal of work has been done, and three main theories have been propounded as to the underlying mechanism. These are the toxaemic, nervous and blood loss theories.

The primary features of traumatic shock are low blood pressure and a reduction of the circulating blood volume. A rapid pulse rate with diminished pulse volume also occurs. According to Gaddum and Dale (1936) the release of histamine or some other similarly acting substance from injured skin can well account for part of the production of such a condition provided

damage occurs over a large enough area.

The various theories will now be discussed.

(1) Toxic theory: - This theory was introduced by Quenu (1918) and by Cannon and Bayliss (1919). They believed that the symptoms following injury to tissues, could be attributed to the release into the circulative blood of toxins from the injured area. Experiments were done on cats, in which trauma to the hind limbs produced fatal shock. They found that section of the nerves prior to infliction of the trauma was without effect, and further that the fluid loss by haemorrhage was insufficient to account for the degree of shock.

At the same time Dale and Laidlaw (1919) noted the striking similarity between histamine shock and traumatic shock. Both were due in the main to a diminished blood volume and a drop in blood pressure. It was assumed therefore that since histamine shock resembled traumatic shock so strikingly, histamine must in part at least be responsible for the symptoms of traumatic shock.

This theory was very popular until 1927 when Smith made the criticism that there were no depressor substances in the blood coming from a traumatized limb when collected during the onset of shock. This finding has since been corroborated by many observers (Blalock 1930; Parsons and Phemister 1930; Holt and MacDonald 1934; Roome and Wilson 1935; O'Shaugnessy and Slome 1935; Dragstedt and Mead 1937).

Parsons and Phemister (1930) further observed that a dog whose blood pressure had been lowered to 60 mm Hg by the action of histamine was quite resistant to haemorrhage, 600 cc being removed before death occurred. In traumatic shock however where the blood pressure was lowered, only 200 cc haemorrhage was sufficient to cause the death of the animal.

O'Shaugnessy and Slome (1935) carried out experiments in dogs in which the blood of one animal was dialysed against that of a second animal, so-called 'vividialysis'. In such a preparation, any dialysable substance in the blood of one animal will pass from its circulation into that of the second animal. They then traumatized a limb of one dog in the usual manner. This dog (A) went into shock, where dog (B) evinced no symptoms. If however histamine was injected into the circulation of dog (A) producing a drop in the blood pressure of that animal, a drop was seen to occur in the second animal (B) indicating that the histamine had passed from one circulation to the other by dialysis.

Using the same methods for the detection of histamine as they had in the experiments on anaphylaxis in the dog, Dragstedt and Mead (1937) were unable to note the appearance of histamine in the blood of the dog in traumatic shock. They further noted that if sufficient histamine were injected slowly intramuscularly to imitate the conditions of traumatic shock, it was easily detectable in the blood of the animal.

Recent observers, using the method of Barsoum and Gaddum (1935a) have been unable to note any difference in the histamine content of blood coming from the limbs of animals before or after the production of traumatic shock. (Holt and MacDonald 1936; MacDonald and Woolfe 1938). Minard (1937b) observed an increase in the histamine content of blood from the traumatised limb of the dog as compared with the normal.

For these reasons, many workers have rejected the histamine theory as a basis for the production of traumatic shock.

(2) The nervous theory of traumatic shock: - In 1921 Crile came to the conclusion that continued stimulation of the sensory nerves would produce shock by exhaustion of the vasomotor centre. Similar theories have been advanced by more recent workers. Simonart (1930) performed a series of

experiments on dogs in which trauma was inflicted to an extremity. He noted that shock occurred only if the nerves to the limb were left intact. If the vessels to the traumatized limb were clamped, shock still occurred. Similar results were reported by Hoet (1929). They came to the conclusion that the main factor in the production of traumatic shock was a stimulation of the nerves from the traumatized area.

These results have been confirmed by O'Shaugnessy and Slome (1935) who performed cross circulation experiments in dogs. The limb of one animal (A) (recipient) was completely separated from the circulation by tying off all the vessels, and it was then perfused by a second animal (B) (donor) by anastomosis of the carotid and jugular to the femoral artery and vein of the limb. They noted that trauma to the limb of dog (A) which was excluded from its own circulation, caused death of that animal. The dog (B) which was perfusing the damaged limb was not affected. They attributed the onset of shock to the production of nervous impulses in the traumatized limb. In further experiments, they perfused the limbs of dogs by means of a Dale-Schuster pump and produced the same results. If they sectioned the nerves before applying the trauma, no shock was observed. Further observations have been made of these nervous impulses using the cathode ray oscillograph (Slome and O'Shaugnessy 1938).

Bell, Clark and Cuthbertson (1938) also noted that the presence of an intact nerve supply to a traumatized limb hastens and increases the severity of shock.

(3) Haemorrhage: - The decrease in blood volume is held by many observers to be due to a localized extravasation of blood and plasma into the traumatized zone and adjacent soft tissues (Smith 1927; Blalock 1930; Parsons and Phemister 1930; Holt and MacDonald 1934) and not due to a generalized filtration

out of the vessels.

In their original experiments, Cannon and Bayliss (1918) came to the conclusion that the loss of blood and plasma which occurred into the traumatized limb of an animal was not sufficient to account for the degree of shock.

Recent observers however have repeated the work of Cannon and Bayliss (1918). Blalock (1930) showed that their method of computing the loss of blood was inadequate in that they neglected the transudation into the soft tissues of the thigh. If the actual amount of fluid loss into both the traumatized limb and into the soft tissues of the thigh is measured, its volume is great enough to account for the death of the animal. These observations were confirmed by other workers. (Holt and MacDonald 1934).

In their experiments on cross circulation, O'Shaugnessy and Slome obtained different results. The donor animals did not die of shock although they supplied blood to and received blood from the traumatized limb of the recipient animal.

Bell, Clark and Cuthbertson (1938) however using a slightly different technique to that used by Slome and O'Shaugnessy found that donor animals died under these circumstances, whereas the condition of the recipient animal was not affected. They do not explain the difference between their results and Slome and O'Shaugnessy with the exception that a different type of anaesthetic was used.

(4) Additional theories:- In an excellent review, Moon (1937) has made a critical survey of the work on shock, and has tried to correlate the symptoms of shock with the pathology. He states also that one must make a distinction between haemorrhage and shock, and recommends the study of the haemoconcentration rather than blood pressure. The pathological picture of shock differs from

that of haemorrhage. According to Moon (1937) shock is characterized by the following: The superficial veins are collapsed and bloodless. The blood in the heart and large vessels and in the parenchyma of organs is dark and thick and has failed to clot. Serous surfaces are diffusely congested and appear cyanotic. The bowels are atonic and the vessels along the mesenteric attachment are engorged and prominent. All the mucous membranes are congested and frequently contain ecchymoses. The lungs are intensely congested either diffusely or in scattered areas. Microscopically the capillaries are distended and the venules are dilated and packed with corpuscles and haemorrhage from the capillaries are numerous. Marked oedema is present. The liver and kidneys are deeply congested and blood oozes and drips from the parenchyma when sectioned.

This picture differs markedly from that produced by haemorrhage, where the viscera are pale and dry, and the abdominal organs do not drip blood when they are sectioned.

Moon and Morgan (1936) showed that marked increase in capillary permeability occurs when shock develops as indicated by haemoconcentration. Moon and his associates (1937) further observed similar congestive, oedematous and haemorrhagic conditions of the viscera following shock in man. Similar observations have been reported by Eppinger (1934) and Eppinger, Kaunitz and Popper (1935).

It is of interest to note that such pathological changes are observed in animals when histamine shock is produced. O'Shaugnessy and Slome (1938) state as one of the reasons against the histamine theory of shock, that such a pathological picture does not occur following traumatic shock. In their animals, the organs are pale and anemic and do not drip blood. According to the view of Moon (1937) this may be attributed then to a failure to distinguish between haemorrhage and shock.

According to Moon many diverse agents may affect the minute vessels with the production of capillary atomy, which results in the pathologic changes characteristic of shock. He believes that certain factors cause a decrease in blood volume with reduced volume flow, deficient delivery of oxygen with resultant capillary atony again so that a self-perpetuating mechanism or vicious circle is produced.

Karady (1934) believed that the symptoms of post-operative shock might be due to an increased sensitivity of the organism to histamine, assuming that histamine were liberated during surgical trauma. Such a supposition is not unreasonable when one considers that sensitivity to many different agents exists in human beings. In an endeavour to devise a means of determining whether a patient was hypersensitive to histamine he found that the response to an intravenous injection of 0.005 mg of histamine in patients was followed by changes in the blood pressure. Four main types of blood pressure response could be distinguished. These have been described in a previous section (see p.48). It was noted on analysis of the individuals examined by this test that those exhibiting a reaction of the second type were prone to go into surgical shock following a major operation. Rusnyak, Karady and Szabo (1935) noted further that post-operative collapse could be prevented in such patients if they were given a course of histamine injected intramuscularly for one week prior to operation.

Further substantiation of this work by animal experimentation was carried out. Rusnyak, Karady and Szabo (1935) performed laparotomies on dogs, one group of which was pretreated with histamine, whilst another of which served as controls. Changes in the blood volume were followed. A decrease of from 32-66% in the blood volume was observed in the controls, whereas in the pretreated group, the greatest decrease was 8% and in some there was even an increase. This has since been repeated on rabbits, using the Gibson and

Evelyn micromethod (1937) for blood volume determination with the same results (Karady 1938). Although the present opinion amongst workers on the problem of shock is away from the histamine theory, it does not seem unlikely, in the light of the above observations, that it may play an important role in the production of shock.

G. Role of Histamine in Burns and Scalds:

As in the case of other forms of cell injury, Lewis (1927) postulated a release of 'H' substance from the cells of the skin. Harris (1927) noted that scalding of the skin of the cat caused no immediate change in the histamine content of the skin, but the histamine content was markedly decreased after the formation of oedema. He inferred from this that no new histamine had been formed, and that the histamine which had been present in the skin was now released into the circulation.

Bennet and Drury (1931) found that both histamine and adenosine-like substances were released from the isolated heart of the cat by burns. They believed that not one, but several toxic substances entered into the production of shock.

Simonart (1930) distinguished between traumatic shock and the shock which occurred following burns, in that he believed the former to be due to nervous stimulation set up in the traumatized area, whereas the latter was dependent upon the release of toxins from the burned area.

The increase in the secretion of adrenaline, with decreased adrenaline content of the adrenal glands, as well as the decrease in blood chlorides, and production of duodenal ulcers, all of which may appear following severe burns (Hartman, Rose and Smith 1926) may be accounted for by the action of released histamine (Gaddum and Dale 1936).

Barsoum and Gaddum (1936) and Code and MacDonald (1937) have observed an increase in the total blood histamine in man following severe burns. The increase was noted at about the same time that manifestations of shock began to occur, usually from the fourth to sixth day. After this time, the histamine content of the blood gradually fell to normal over a period of weeks. Barsoum and Gaddum (1936) came to the conclusion that the rise in blood histamine was not due entirely to its production in the burned skin, but that it may also be secondary to pathological changes in the kidney and liver.

Using the method of Yokoyama (1936), Kisima (1938) estimated the histamine content of various organs, and of the blood of dogs which had one-tenth to one-fifth of the skin surface burned. He noted an increase of 7-10 times the normal histamine content of the spleen, liver and pancreas. Histamine appeared in the blood and urine within 6 to 12 hours reaching a maximum in 49 to 96 hours, where it remained for two to three days, and then disappeared. He further observed that removal of the skin from the burned area inhibited this release of histamine.

Rosenthal (1937) obtained a principle from the blood of shoats and guinea pigs which appeared within one hour after the skin of the animal had been burned. This substance resembled histamine only in that it contracted the guinea pig uterus. It was however inactivated when heated to 60°C for 30 minutes.

Recently Bernhard Kreis (1938) has come to the conclusion that histamine is not one of the factors in the production of the shock which follows burning. He made cold extracts of tissues from normal and burned guinea pigs and rabbits. Muscle and skin were mainly used. The extracts were prepared in two ways, firstly by grinding in Ringer solution for 8 days at ice-box temperature, so-called native extracts, and, secondly by gentle

boiling of the tissues in Ringer solution for two hours.

These were filtered and kept sterile. Extracts from normal animals had no effect when injected into animals, whereas injection with extract from burned tissues produced the same pathological changes that are observed following severe burning of the skin. He is of the opinion that the reaction cannot be an anaphylactic one since no sensitisation is necessary, as has been suggested by other observers (Eppinger 1935; Henlein 1935). He further is of the opinion that the changes which are produced by the injection of these extracts do not sufficiently resemble those which occur following the injection of histamine and upholds the view that such toxic manifestations are the result of split-proteins such as peptone and albuminoses.

Bernhard Kreis (1938) examined these extracts for the presence of histamine and could find none. The extracts were not altered in any way, but were simply assayed on the guinea pig ileum preparation. It may be noted here that he found histamine in small amounts in one specimen from guinea pig lung tissue and none from a second. Obviously if no histamine were found in lung of this animal, his method of extraction or assay must be at fault.

In this connection it also is of interest to note that if saline extracts of ground up tissue are assayed before and after being heated, a marked increase in the histamine content of the extract is found after heating (Trethewie 1938). It is believed that heating releases the histamine which may be bound to tissue particles.

H. Histamine as a Cause of the 'Futterrehe' or Hoof-disease in Horses:

In 1934 Ackerblom carried out extensive work on this disease which is characterised by vascular and inflammatory changes in the hoof eventually leading to a loosening and separation of the outer covering of the hoof from the core. The disease is accompanied by general symptoms such as raised temperature, increased pulse and respiratory rates, rigor, colic and

inflammatory changes in the joints.

Ackerblom was able to reproduce the local changes in the hoof as well as many of the accompanying symptoms by means of the administration of histamine. Pathological sections of the hoof show marked similarity.

He was further able to isolate histamine and histamine forming bacteria from the intestinal contents of horses fed with rye. These bacteria are not present in the intestinal contents of all horses. If such animals are fed on rye, the disease will not occur. But if they are given these histamine forming bacteria perorally, production of the disease will occur.

In later experiments (Ackerblom and Sjoberg 1938) the same results were obtained by means of chronic intravenous injections of histamine in horses.

I. The Alarm Reaction:

In 1936 Selye showed by experiments on the rat that if the organism is severely damaged by acute non-specific nocuous agents such as exposure to cold, surgical injury, or intoxications with sublethal doses of various drugs such as atropine, morphine or formaldehyde etc. a definite syndrome appears. Selye divided the syndrome into three stages, the first one of which was called the 'Alarm Reaction'.

The symptoms of this syndrome are independent of the nature of the damaging agent and represent the response of the organism to damage as such.

The first stage develops within 6 - 48 hours after the stimulus has been given and is characterized by a rapid decrease in the size of the thymus, spleen, lymph glands and liver. There is tendency towards oedema formation, a fall of body temperature and the development of acute erosions in the gastro-intestinal tract, in particular the stomach, small intestine and appendix. There is also a loss of cortical lipoids and chromaffin substance from the adrenal glands.

The second stage begins about 48 hours after the initial stimulus has been given and is characterized by marked hypertrophy of the adrenal glands. During this period the animal has acquired a non-specific resistance to damaging stimuli. Thus animals in which an alarm reaction had been produced by one drug became resistant to otherwise lethal doses of another drug. (Selye 1938). This stage has been called the stage of resistance and is believed by Selye to be due to the adaptation of the animal organism.

If treatment with a nocuous agent is continued the animals finally lose their resistance and succumb with symptoms similar to those seen in the first stage. There is a great similarity between the symptoms of adrenal insufficiency and those of the alarm reaction and Selye (1937) has emphasized this fact.

Although definite proof is lacking that histamine is a causative factor in the production of the alarm reaction, certain observations would greatly increase the possibility of this being the case.

In 1936 Karady and Bentsath were able to show that anaphylactic shock in the guinea pig could be prevented by pretreatment with small doses of histamine. In further experiments Rusnyak and Karady (1937) showed that pretreatment of sensitized rabbits with histamine increased and prolonged antibody formation, so that when the antigen was given, no anaphylactic shock occurred.

Recently Karady, Selye and Browne (1938) have shown that by subjecting sensitized guinea pigs to a relatively mild alarm reaction followed by an interval of eighteen to twenty-four rest, the anaphylactic response of these animals to subsequent reinjection of the antigen is decreased.

The observations of Bartelheimer and Afendulis (1938) are very interesting in this connection. They first noted that the production of insulin shock several times during the course of sensitisation of guinea pigs

markedly decreased the severity of the anaphylactic reaction which was induced by giving of the antigen. They noted further that no protection was afforded by the action of insulin alone, i. e. if its 'shocking' action were inhibited by giving glucose at the same time. A study of the blood histamine using the method of Riesser (1937) revealed that a release of this substance occurred at the height of insulin shock. No release was noted if shock did not occur. They concluded that the protection which was afforded by the production if insulin shock was due to sensitisation with histamine.

Since insulin shock may be regarded as an alarming or damaging stimulus, it is easy to suppose that histamine may be liberated during the alarm reaction and may account for part of that syndrome.

Encephalitis:

Recently Spiegel - Adolph and Spiegel (1937) have reported the production of encephalitis in rabbits by the daily intravenously injection of histamine for 14 to 32 days. The dose varied from 0.1 - 0.4 mg/kg.

"The Relationship of the Adrenal Gland to Histamine."

(a) Medulla: - In the early observations on the pharmacological properties of histamine, it was noted that the action of adrenaline was antagonistic to that of histamine (Dale and Richards 1918). If the blood vessels were dilated by histamine, their tone could be restored by the action of adrenaline. Dale (1920) found that the pupil of the cat's eye after denervation (sensitive to adrenaline) dilated following an injection of histamine. He believed that this was due to the release of adrenaline by the action of histamine on the adrenal medulla. It was noted by Kellaway and Cowell (1922) that whereas sweating and dilatation of the pupil of the eye occurred following the intravenous injection of histamine into an intact cat, these symptoms did not occur in an animal from which the adrenal medullae had been removed. Further evidence that histamine stimulated the secretion of adrenaline was furnished by the observations of Burn and Dale (1926) who noted that the effect of an intravenous injection of histamine in cats with a low blood pressure was complicated by a secondary rise following the primary drop in the blood pressure. Increase in pulse rate was noted at the same time. The rise in blood pressure was even greater if the animal was first eviscerated, thereby diminishing the circulating blood volume. If the adrenals were removed, this rise in the blood pressure did not occur. Szczygieslki (1932) injected histamine directly into the cocliac stump of an eviscerated cat and noted a marked rise in the blood pressure.

The antagonistic action between histamine and adrenaline was studied by MacKay (1929) who observed that the secretion of saliva produced by histamine was greatly enhanced by adrenalectomy and decreased by the simultaneous administration of adrenaline.

Perla and Marmorston-Gottesman (1929) were able to increase the

resistance of adrenalectomized rats to histamine by an injection of epinephrine in 50% of cases only. A single injection given subcutaneously protected 2 to 8% only of rats against a lethal dose of histamine. Wyman (1928) on the other hand found that an intraperitoneal dose of adrenaline afforded protection against the action of histamine in adrenalectomized rats.

In 1937 Ingle carried out experiments in which the adrenal medullae were removed and as an added precaution cortin was administered. He found that these animals were less resistant to histamine than were intact animals. He further noted that large amounts of cortin would not raise the resistance of these animals to histamine whereas administration of epinephrine did. He criticised the results of Perla and Marmorston-Gottesman by stating that their animals were in a state of adrenal insufficiency and were improved by the administration of cortin.

It was noted by Schild (1936) that release of histamine from the perfused sensitized guinea pig lungs after addition of the antigen was decreased by the addition of adrenaline to the perfusion fluid.

Wyman and Suden (1934) noted that the hyperglycemia which follows the injection of small amounts of histamine in normal rats did not occur if the animals were previously adrenalectomized. Instead a hypoglycemia was observed and this occurred even when cortical tissue was transplanted in the animal.

Kim (1936) was able to inhibit the production of arteriosclerosis which occurs in rabbits following the daily administration of adrenaline for six weeks by the simultaneous daily administration of histamine. He was unable however to inhibit the production of arteriosclerosis which occurs following the daily administration of vigantol (irradiated ergosterin) by the simultaneous administration of histamine.

(b) Cortex: - In 1924 McCarrison made the observation that injections of histamine caused hypertrophy of the adrenal glands in young rats. Similar

observations have been made not only for histamine but various other toxic substances (Selye 1936). In 1926 Banting and Gairns noted that removal of the adrenal glands in the dog decreased the resistance of that animal to histamine by about thirty times.

Wyman (1928) and Marmorston-Gottesman and Perla (1931) noted that the resistance of the rat to histamine was greatly diminished following adrenalectomy. The sensitivity to histamine reached its maximum point at about ten days after the removal of the glands and was increased to about twenty times the normal. They noted also that the resistance of the animals to histamine could be restored by the administration of cortin. In 1929 Wyman noted that the severity of the symptoms of anaphylactic shock in the rat were greatly increased after adrenalectomy. Since the same effect was noted even 180 days after adrenalectomy and cortical tissue had regenerated, he was of the opinion that the increase in susceptibility was due to the lack of medulla rather than of the cortex. Administration of adrenaline however before giving the antigen did not have any effect.

The observations of Selye (1937) are interesting in this connection. He has likened the third or exhaustion stage of the general adaptation syndrome to one of adrenal insufficiency. Indeed, adrenalectomized animals are much less resistant to damaging agents and develop an alarm reaction with much smaller stimuli than do normal animals.

It is also interesting to note that Clark and MacKay (1939) were able to inhibit the production of hemoconcentration and decrease in plasma sodium which occurs in adrenalectomized rats following muscular exercise by pretreatment of the animals with histamine up to the time of the adrenalectomy.

"The Relationship of Vitamin 'C' to Histamine."

Hochwald (1935) noted that anaphylactic shock could be inhibited in the sensitized guinea pig by the administration of three intraperitoneal doses of ascorbic acid, the first two hours and the last ten minutes before giving the antigen. Peroral administration was without effect. No effect was noted if two hours elapsed between giving the vitamin and the antigen, nor was any protection afforded if the vitamin was given before sensitisation. Hochwald noted further that ascorbic acid had no effect on histamine shock. He therefore postulated that the mechanism of the action of vitamin C in anaphylaxis was to prevent the liberation of histamine rather than to inhibit or inactivate the action of histamine after its liberation.

In further work Hochwald (1936) observed that similar protection might be produced by the administration of glutathion, cystein and other sulphohydrogen compounds. He concluded that the mechanism of action ascorbic acid and these other substances on histamine was one of reduction since all of these substances have the common property of being reducing agents.

The observations of Hochwald (1935) were supported by other workers (Lemke 1936; Solomonica 1936; Giroud and Giroud 1936).

Ungar, Parrot and Levillain (1937) reported some in vitro experiment which tended to confirm this hypothesis. They were able to inhibit the release of histamine by an antigen from the isolated tissues of a sensitized guinea pig by the addition of vitamin C to the fluid containing the tissues. It was noted that this inhibiting of the histamine release occurred only if the vitamin C were allowed to remain in contact with the tissues for at least twenty minutes before adding the antigen.

The results of Hochwald (1935) could not be confirmed however by other workers. (Schwartz and Cisloghi 1935; Van Niekerk 1937).

Recent work on dogs also indicates that vitamin C does not inhibit the release of histamine by the antigen-antibody reaction (anaphylaxis). Eyer, Dragstedt and Arellano (1938) produced anaphylactic shock in a group of dogs. They gave 250 mg of cevitamic acid daily by mouth to four to these animals during the period of incubation (15 - 20 days). To another five dogs they gave a daily oral dose of 500 mg during the incubationary period and an intravenous injection of 500 mg just before giving the antigen. Finally in nine dogs, an intravenous injection of 100 mg/kg of the vitamin was administered during the last forty-five minutes before giving the provocative injection of horse serum. No difference in the severity of the anaphylactic shock produced in any of these animals was noted as compared to that produced in control animals.

These same workers have also produced evidence which indicates that vitamin C does not inhibit the release or action of histamine, when it is released in the organism by a mechanism other than the antigen-antibody reaction (Dragstedt, Eyer and Arellano 1938). They produced peptone shock in dogs which were pretreated with intravenous cevitamic acid and in control animals. No difference in the severity of the shock produced in either group was observed.

Perla (1936) was unable to note any difference in the resistance of adrenalectomized rats to histamine after pretreatment with ascorbic acid from those which had not received the vitamin. He used much smaller doses (10 mg daily for eight days) than Hochwald (1935) who gave three injections of 100 mg of ascorbic acid each.

The work of Holtz (1937) has been reviewed in a previous section (p.13) He was able to show that histamine may either be formed from histidine by the action of vitamin C or that histamine itself could be inactivated by the vitamin.

"Histamine Sensitivity and Histamine Resistance."

In the discussion of histamine sensitivity, a distinction should be made between sensitivity of cells to histamine as such and an increased sensitivity of tissue cells to some specific agent so that they liberate histamine more readily when exposed to that agent.

It is true for instance that certain individuals may become sensitive to foreign proteins, and the resulting interaction of the antigen and antibody in the cell causes a release of histamine. Similarly in those patients, who manifest dermatographia, the cells of the skin are sensitive to slight stimulation and a release of histamine is the result of such stimulation, The same stimulus, i. e. scratching the skin produces the usual mild response characterized by reddening, but does not result in the formation of a wheal. Many other types of sensitivity may be cited such as that to cold, heat etc. These patients however do not manifest any deviation from the normal response to a subcutaneous injection of histamine.

Nevertheless, the reaction to either an intravenous or subcutaneous injection of histamine varies in different individuals, and marked reactions have known to occur from small doses (Karady 1934).

Ramirez and George (1929) noted that patients suffering from asthma gave increased responses to intracutaneous injections of histamine. Rusnyak, Karady and Szabo (1934) noted that patients who manifested the signs of surgical shock following major operations gave a typical response to an intravenous injection of histamine prior to the operation, indication hypersensitivity to histamine.

It would seem that the state of histamine sensitivity or resistance of an organism as the case may be is related to certain endocrine glands,

namely the adrenal and the thyroid. The role of the adrenal gland has already been discussed in a previous section. (p. 114).

That the thyroid may play a part in resistance against histamine has been suggested by the observations of Karady (1934). He noted that the response to an intravenous injection (0.005 y) of histamine was greatly diminished in patients with hyperthyroidism. Instead of the usual drop of 20 mm very little change occurred in the blood pressure. He further observed (1936b) that if such patients had their thyroids removed, or if they were treated either by X-ray or with iodine, the usual response to histamine was more marked and that now the blood pressure dropped at least 20 mm following an intravenous injection of 0.005 mg of the amine.

It was also noted (Karady 1936b) that if cats were treated with thyroxin, the response of the blood pressure was altered. Normally, a drop in the blood pressure of the cat follows upon the intravenous injection of histamine. After treatment with thyroxin for ten days, a rise is observed instead.

This may be explained by the fact that the capillaries of the skin are constantly dilated in cases of hyperthyroidism. Since the drop of blood pressure after histamine occurs mainly by dilatation of the capillaries, it seems obvious that histamine cannot exert its action.

Finally, the resistance imparted to an animal by histamine pre-treatment must be considered. It has been observed by many workers, that an animal which receives increasingly large doses of histamine either subcutaneously or intravenously will become more resistant to histamine. (Eichler and Killian 1931; Jacobs and Mason 1936; Karady 1936a; Selye 1937).

The actual mechanism of this reaction is not understood, although various theories have been advanced. It has been known for some time that if a cat is given a large dose of histamine so that it is precipitated into histamine shock, the blood pressure response to a small dose of histamine will

be reversed, that is a rise occurs (Meakins and Harington 1922; Feldberg and Schilf 1930). This action is attributed to the fact that the capillaries are dilated, and additional histamine acts therefore mainly on the arterioles which it constricts in this species.

It was noted (Karady and Bentsath 1935) that pretreatment with histamine diminished the sensitivity of patients to histamine and afforded protection against surgical collapse. In order to determine the mechanism of the desensitisation, Karady (1936a) desensitized cats to histamine by pretreatment with the amine. Whereas a drop of blood pressure is produced ordinarily by the intravenous injection of 10 y of histamine, this did not occur in the pretreated animal, and a rise was observed instead. Kokas, Sarkady and Went (1937) were unable to confirm these results using the dog. However it had been previously observed (Eichler and Killian 1931) that whereas 2-3 mg would cause the death of a rabbit when injected intravenously this animal would tolerate a dose of 150 mg, if the injections were given in rapid succession, increasing the size of each subsequent dose.

Rusnyak, Karady and Szabo (1936) further showed that this pretreatment with histamine diminished surgical shock in the dog. A study of the blood volume at the same time revealed that whereas a marked diminution in the circulating blood volume occurred in the non-treated animals, very little decrease or even an increase was observed in the pretreated animals.

This was confirmed again by Karady (1938) who showed that pretreatment with histamine prevented the decrease in the blood volume usually observed after either histamine or surgical shock in rabbits. He used the micromethod of Evelyn and Gibson (1937) for the blood volume determinations.

Recently MacKay and Clark (1938) have shown that pretreatment of adrenalectomized animals with histamine will protect these animals against the effects of nephrectomy. In further work they have shown that pretreatment of rats with histamine for a period of eleven days prior to adrenalectomy will

protect these animals against symptoms such as decreased blood volume, lowered serum sodium etc. which occurs in adrenalectomized animals subjected to muscular exertion (Clark and MacKay 1939).

No effects however were observed on the performance of muscular work, the level of the liver or muscle glycogen or blood sugar or the toxic effects of hypertonic glucose solutions administered per os to adrenalectomized animals.

A tolerance to histamine could not be developed in the guinea pig by daily subcutaneous injections of 0.1 mgm per 100 grams body weight over two weeks, or by increasing doses according to these observers. The survival time after 0.5 - 0.6 mgm per 100 gms body weight in such pretreated animals was not greater than in that of the controls. They used histamine diphosphate (30% histamine base).

Smith (1939) on the other hand was able to produce a tolerance to histamine in guinea pigs. He found the approximate minimum lethal dose in untreated animals to be 0.32 mgm per kilogram of histamine dihydrochloride given subcutaneously (60% histamine base). By pretreatment with gradually increasing doses over a period of two to three weeks, he was able to increase the tolerance 500 - 600%.

Smith (1939) was also able to protect sensitized guinea pigs from going into anaphylactic shock after administration of the antigen by pretreatment with histamine during both the pre-sensitisation and post-sensitisation periods. Of twenty animals treated by this method only one died.

The difference in the results of Clark and MacKay (1939) and of Smith (1939) may perhaps be attributed to the fact that the former workers gave such large doses of histamine in order to shock the animals. In terms of the hydrochloride salt, they administered 2.5 mgm per kilogram, whereas Smith found that the M. L. D. of untreated animals was 0.32 mgm per kilogram.

Farmer (1939) has shown that the 'desensitisation' which pretreatment with histamine produces, exerts a defence mechanism not only against histamine but against the anaphylactic reaction. He believes therefore that the desensitisation is 'non-specific' in nature. This was demonstrated by noting the action of histamine and of a specific antigen on isolated uterine strips taken from (a) animals which had been pretreated with histamine, and (b) animals which had not received histamine. Since the contraction of the sensitized uterine strips upon addition of the antigen depends upon a liberation of histamine, it seems that the desensitizing action is a specific one in that the contraction of the uterine strips is due to histamine in both cases.

Selye (1937) has shown that the acute appendicitis which may be produced in rats by a large intravenous dose of histamine may be prevented if the animals are first pretreated with histamine.

It has also been observed by Howlett and Browne (1937) that the water retention which may be produced in rats following the intravenous injection of histamine may be greatly diminished if the animals are first pretreated with histamine.

It is interesting to note that Selye (1937) has been able to confer non-specific resistance to animals by exposing them to an alarming stimulus. The animal becomes resistant about twenty-four hours after exposure. This has been shown by the experiments of Karady, Selye and Browne (1938) in which the anaphylactic shock induced in sensitized guinea pig by the giving of an antigen was markedly inhibited if the animals were exposed to an alarming stimulus twenty-four hours before receiving the antigen.

EXPERIMENTAL WORK.

The experimental work which is about to be described is concerned with the mechanism of the destruction or inactivation of histamine and with relationship of the adrenal gland to histamine metabolism. A description of the methods of extraction and assay will first be presented.

"Methods of Extraction."

(a) Blood: - The modification of the method of Barsoum and Gaddum (1935a) as described by Code (1937b) was used in the extraction of blood. Since most of the experiments were carried out on the rat, it was impossible to obtain 10 cc of blood consistently. It was therefore decided to use samples of 5 cc routinely. In all of the experiments whole blood was used. The method with the modification of the amounts of the various reagents used is as follows: Five cc of whole blood is placed into eight cc of 10% trichloracetic acid, making sure that it is well mixed to avoid clumping, and is allowed to stand for 45 to 60 minutes in a small 50 cc Erlenmeyer flask. It should stand not less than 30 minutes in order to allow the proteins to precipitate completely and not longer than 90 minutes in order to avoid adsorbtion of histamine on the precipitate. The contents of the flask are then filtered through a small Buchner funnel by suction into large 40 cc centrifuge tubes and the precipitate is washed four times with 4 cc portions of 10% trichloracetic acid. The filtrate which should be clear is transferred to large Erlenmeyer flasks (250 cc) and 5 cc of concentrated HCl added. This is then boiled gently using a micro-burner

for 90 minutes, excess evaporation being prevented by placing a reflux air-condenser in the top of the flask. This consists of a glass tube about 1 cm in diameter and about 65 cm in length which is fitted into the neck of the flask, both ends of which are left open. Care should be taken to prevent desiccation by the addition of water, but this will not be necessary in most cases as the volume is generally decreased only by about two-thirds. After the acid hydrolysis, the extract is transferred to small round-bottomed pressure flasks (200 cc) care being taken to transfer all the extract by successive washings with distilled water. It is now evaporated to dryness in vacuo in a water bath at a temperature of about 90°C. In order to speed up the evaporation and to prevent bumping, the tip of a thin glass tube drawn out to capillary fineness is placed so that it just reaches to the bottom of the flask. It is fixed from the top of the still arm to which the evaporation flask is attached. When evaporation is complete, two successive 10 cc portions of 96% alcohol are added and evaporated off in order to remove the excess acid. The apparatus used for evaporation of both blood and tissues has been arranged to evaporate six flasks at once. Suction is provided by a high-pressure water pump. This apparatus must be thoroughly cleaned since small amounts of histamine may adhere to the rubber joints at the bottom of the still tube. Such amounts might make considerable difference in a blood histamine assay. It has been the practice to reserve three of the tubes for blood specimens and to use the other three for tissues. Two cc of distilled water are added to the flask and the residue is taken up. This is greatly facilitated if the flask be kept warm. After one or two minutes, this is filtered by suction through a small filter paper of about 1 cm in diameter in a Buchner funnel into a small graduated centrifuge tube of 15 cc capacity. The procedure is repeated with two more 1 cc portions of water to make sure that all the residue has been removed. The filtrate is now neutralized with

N/5 NaOH. Usually 0.5 - 0.6 cc suffices. It has been found that neutral litmus is an adequate indicator. Following neutralisation, the extract is made up to the original volume of the blood with distilled water, namely 5 cc and is now ready for assay. The extract frequently becomes turbid following neutralisation, but this does not in any way affect the result of the assay. A series of determinations were performed on duplicate specimens of blood and the figures obtained in the two series were within 0.5%. Similarly histamine added to specimens of blood were recovered within 95 - 98%.

(b) Tissues: - The method used for the extraction of histamine from tissues is essentially that described by Best and McHenry (1930). It is carried out as follows: 20 cc of 10 HCl is placed in small weighing bottles with ground glass tops, with a fluid capacity of about 100 cc. The tops are replaced and the flasks are weighed.

The freshly removed tissue, generally between 1 - 3 gm in weight is placed directly into a bottle containing the acid and the top is again replaced to prevent evaporation. The whole is then again weighed. The difference between the first and second weighings give the weight of the tissue. The bottle is then placed in a water bath at a temperature of 98°C for one hour. It is then removed and the contents are washed into a round bottomed pressure flask of 200 cc capacity. The contents are first broken up when necessary, using a glass rod, then evaporated to dryness in vacuo at an external temperature of about 80-90°C. The addition of two or three small glass beads facilitates evaporation and prevents bumping. When evaporation is complete, two successive portions of 96% alcohol, 10 cc each, are added and evaporated off to remove the excess acid. The residue is then taken up in 5 cc of normal saline and transferred to a small beaker. Two additional washings (5 cc each) with normal saline are carried out. The residue on the sides of the flask may sometimes be difficult

to remove and this may be facilitated by scraping the flask with a flattened glass rod. The flask should be kept warm while this last procedure is carried out.

The contents of the beaker are then neutralized first with a few drops of 20% NaOH and then with N NaOH. Neutral litmus is used as the indicator. At this point, the extract has a muddy brown appearance. It is then filtered by suction in a Buchner funnel and the residue is washed with three portions of 5 cc each of normal saline. The final volume of the extract is then made up to 50 cc in volumetric flasks by the addition of 0.9% sodium chloride solution. When smaller amounts of tissue are used or when the quantity of histamine is small, the technique may be modified so that the final volume is 20 or 25 cc. The final extract should be a clear brown solution.

Some of the final extracts may have a turbid appearance and these have generally been difficult to filter. They are occasionally toxic to the guinea pig gut preparation in that relaxation of the intestine may be delayed by their action. They have however no toxic effect using the cat blood pressure method of assay.

"Methods of Assay."

(1) Guinea Pig Ileum Preparation:

The use of the guinea pig ileum as a test for the quantitative assay of histamine was described by Guggenheim and Loeffler (1916).

In principle the intestinal preparation is suspended in a small bath containing Tyrode solution, with constant oxygenation and at a temperature of 38°C.

(a) The apparatus: - The apparatus which has been used is a modification of the usual constant temperature bath. Certain changes have been introduced in order to facilitate the assay of extracts.

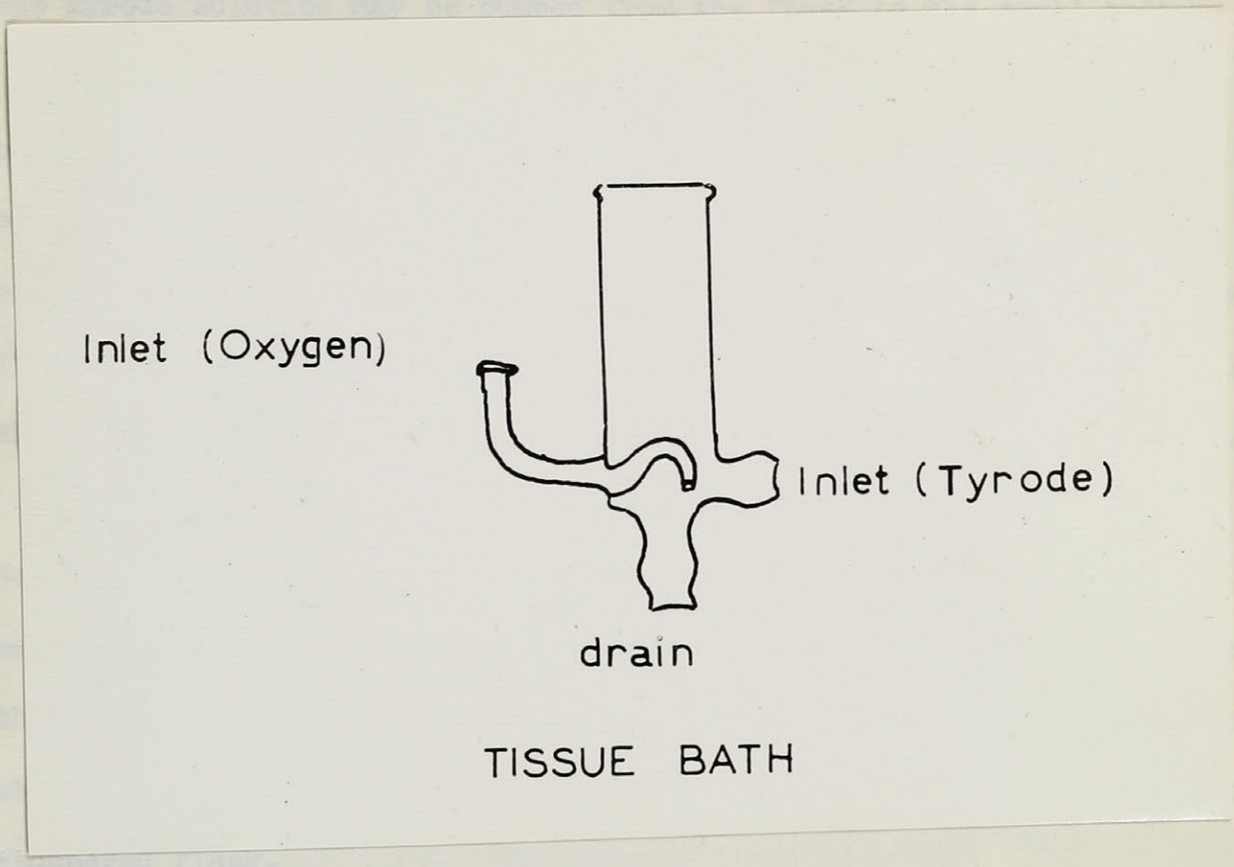
The bath measures 12 x 8 inches and is six inches deep. The water level is kept at a constant height of four inches by a constant water leveler and the water is maintained at a constant temperature of 37°C by means of 100 watt electric bulb, controlled by a mercury thermostat.

The bath containing the intestinal preparation has a fluid capacity of 2.5 cc and is supported in the large bath so that the water level in the outside bath reaches to about one sixteenth of an inch of the top of the tissue bath. It has an inlet and an outlet for the perfusing solution, and an oxygen inlet (Fig. 1). A thread is kept around the hook of the oxygen inlet with both ends fixed in plastacine on the side of the large bath. This greatly facilitates the insertion of a piece of intestine. One thread from an end of a piece of intestine is tied to one of the threads from the bath, and by gently pulling the other one, the end of the gut is pulled down to the bottom of the tissue bath and fixed there. The thread from the other end of the intestine is attached to the short end of the writing lever, which is located directly above it (see Fig. 2).

The writing lever should be balanced so that the writing end is slightly heavier than the end to which the intestine is attached, and this is easily accomplished by placing some plastacine at the short end. This too facilitates the attachment of the thread from the intestine.

In order to maintain the fluid of the tissue bath at a constant level, a small capillary tube bent to hang over the side of the bath containing the intestine is placed into position. If the tissue bath is filled to overflowing each time, it automatically adjusts itself within 15 seconds to the level of the large bath.

Figure 1.



The Tyrode (perfusing) solution is placed in 100 cc amounts into Erlenmeyer flasks with a 200 cc capacity. These (2 or 3) are then placed directly in the large bath which keeps them at 37°C and are weighed down by heavy lead wire which keeps them in position. By means of an atomiser bulb, the Tyrode solution may be pumped from the flask to the small bath.

The fluid inlet and outlet of the small bath are controlled by means of two spring clamps on the rubber tubing. They are mounted on bases which rest on the bottom of the bath and their upper levers are extended so that they project above the water level, facilitating operation. When one flask of Tyrode solution is emptied, the stopper of another is removed and replaced by that containing the glass tube leading to the small bath (see Fig. 2).

Tyrode solution has the following composition:
NaCl 8.0; KCl 0.2; $CaCl_2$ 0.2; $MgCl_2$ 0.1; NaH_2PO_4 0.05; $NaHCO_3$ 1.0; Glucose 1.0; made up to 1000 cc with distilled water. The salts must be well dissolved before they are mixed since the solution will become turbid if this precaution is not observed. Such a solution will keep well for a month if it is kept in a glass stoppered flask.

It has been found that Tyrode solution is a much better medium if it is allowed to stand for three to four days before using, although it may be used immediately after making up.

A kymograph running at a very slow rate is used for obtaining the tracings. The time-marker should indicate thirty-second intervals.

(b) Preparation of the Intestine:

A guinea pig, preferably one that has been starved for twelve hours, is killed by a blow on the head, the abdomen is opened and the ileum is rapidly removed, about 20 cm in all. If the animal has been starved the gut is easily emptied by gentle milking. Occasionally however it is difficult to empty because of contracted areas. The distal (6 - 7 cm) portion is discarded, and the

Figure 2

Apparatus for the Assay of Histamine.

remaining portion is placed in a beaker containing Tyrode solution.

A small piece of the distal end of the kept portion is then removed and placed in a flat dish containing Tyrode solution. This corresponds to the intestine found about 10 cm from the appendix. The portion should be about 2 cm in length. Threads are tied to both ends, one of which is later tied to the thread leading to the tissue bath. The gut may now be pulled into the bath, supporting it by the other thread which is eventually fastened to the writing lever. This procedure is facilitated by transferring the gut to a small beaker of Tyrode solution which is placed in the large bath near the tissue bath.

After the intestine is in position, in the small bath, it usually takes about 15-20 minutes before it becomes sensitive to histamine. Sometimes the addition of a small amount of a 1 y solution of histamine which causes a maximal contraction will speed up the onset of sensitivity. It should be washed out immediately after the contraction has occurred.

It has been noted that the addition of atropine in amounts sufficient to inhibit contraction by acetyl-choline in 0.5 y doses, does not affect the response to histamine, and further enhances the assay by inhibited the small spontaneous contractions which normally occur. Atropine has been added to the Tyrode solution routinely in a concentration of 1×10^{-7} (1 in 10 000 000).

(c) Method of Dosage and Preparation of Standard Solutions:

Assay by this method is based upon comparison of the height of a contraction produced by an unknown solution of histamine with that of a standard histamine solution.

Standard solutions of histamine are prepared by weighing out 16.6 mg of histamine dihydrochloride which is equivalent to 10.0 mg of histamine base since the dihydrochloride salt is only 60% histamine. This is then diluted to 100 cc with distilled water making a concentration of 0.100 mg/cc or

100 y/cc (1 y = 0.001 mg). One cc of this solution is then diluted 100 times with 0.9% NaCl giving a 1 y/cc solution. The same glassware is always used for making up the standard solutions. The 100 y/cc solution may be kept for a week if it is stoppered and placed in the ice-box. The usual precaution of raising the temperature to 20°C before measuring it should be observed.

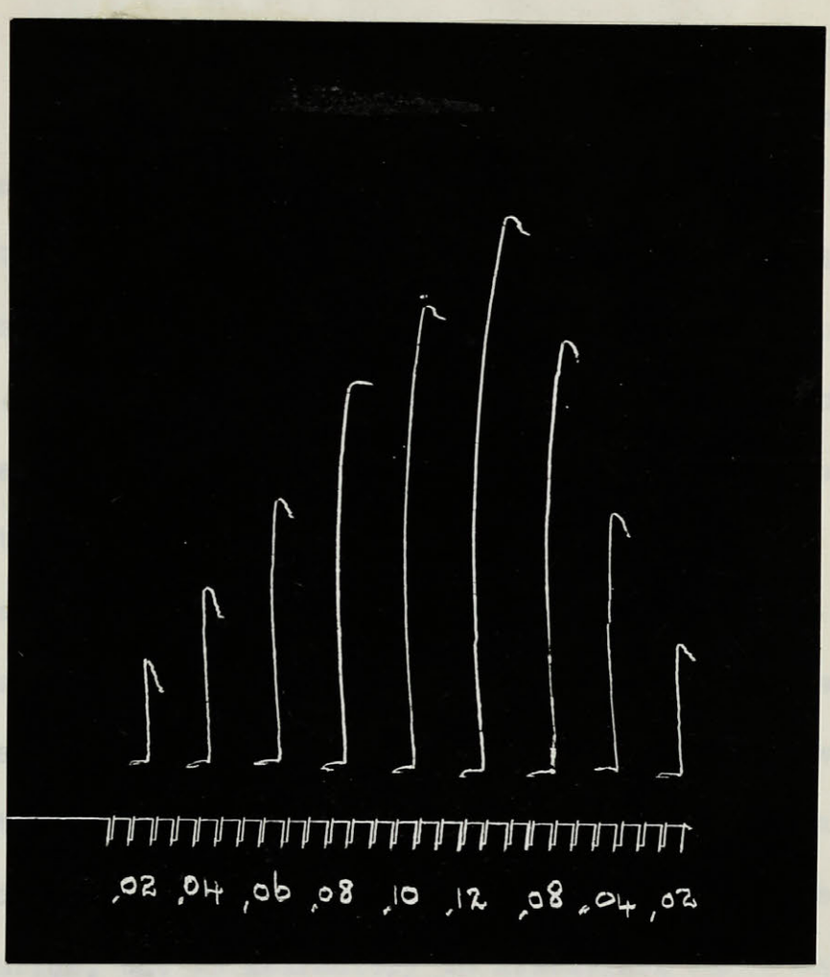
Finally using N saline six standard solutions of 20 cc each are made up from the 1 y/cc standard of the following strengths: 0.02y, 0.04 y, 0.06 y, 0.08 y, 0.10 and 0.12 y/cc. In carrying out an assay, the same volume of fluid is added for the standards and the unknowns. The volume is measured in a graduated one cc glass syringe of the O. T. type. A glass tip is affixed by small rubber tubing. The volume is equal to 0.05 cc of fluid in the syringe plus the quantity in the glass tip (± 0.05 cc) or about 0.1 cc in all. Thus the volume of fluid added to the bath is always the same. This avoids the necessity for minute measurements by a micro-syringe, and as a result the values may immediately be read from the tracing. This volume of distilled water or normal saline which has been made slightly acid or alkaline, does not have any effect upon the gut preparation, when added to the tissue bath.

(d) The Assay of Histamine:

As soon as the gut preparation responds to a dose of histamine of 0.1 y/cc it is ready for use. It is always best to repeat this dose several times as the preparation gains in sensitivity until the maximum is reached. After this the sensitivity varies somewhat, but this variability is equally effective on both standards and unknowns so that it may be ignored.

Such a preparation is sensitive to differences of 0.005 y of histamine. Figure 3 shows the response of the gut to various standards. It will be noted that the sensitivity of the gut has slightly increased towards the latter part of the tracing since the 0.08 y and 0.04 y standards now stimulate a greater response than they did in the first portion of the assay.

Figure 3.



Showing the Sensitivity of the Gut-Preparation to Different Standard Solutions of Histamine (Time in 30 Seconds).

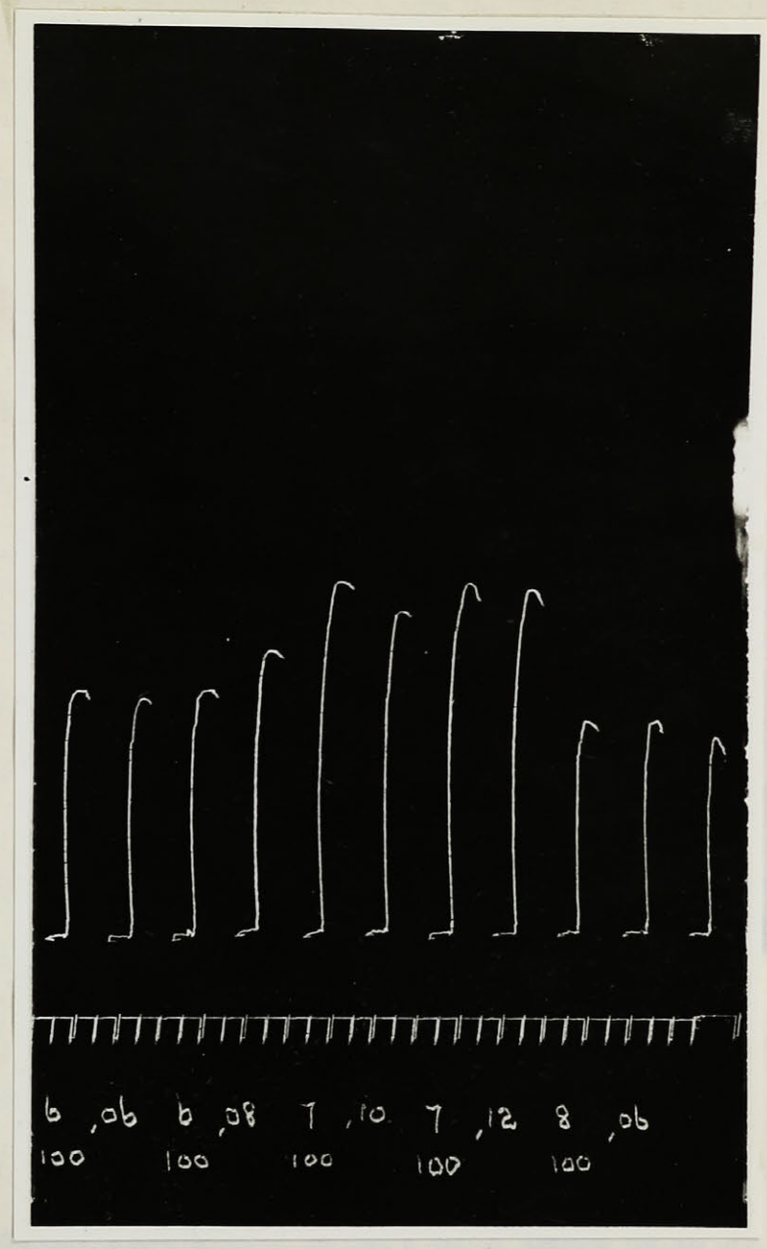
Satisfactory results have been obtained by timing the assays as follows: The histamine solution is placed in the tissue bath and allowed to act for 30 seconds. The contraction to histamine is usually complete by 15 seconds. The lever is then moved away from the drum and the tissue bath is drained, and then filled with fresh Tyrode. At the next 30 second mark, the lever is replaced on the kymograph and 30 seconds afterwards another dose of histamine is placed in the bath. Thus the gut contracts once every 90 seconds. Such a preparation may last as long as two hours with this regular stimulation. It is best to alternate an unknown with a standard solution. A typical assay may be seen in Fig. 4. The standard solutions are always indicated by decimal numbers, i. e. 0.1, 0.04 etc., whereas the unknowns are designated by a whole number, frequently with the dilution marked below, as is usually required in the assay of tissues.

Barsoum and Gaddum (1935a) demonstrated that if a guinea pig gut preparation is stimulated by a large dose of histamine, i. e. 50 y/cc, a maximal contraction occurs, and the intestine takes about twenty minutes to relax even after several washings. When complete relaxation has occured, the preparation will respond to other stimulants such as choline or acetyl-choline or barium chloride, but is refractory to histamine. This may therefore be used as further evidence that an unknown substance is histamine. An example is seen in Fig. 5.

(2) Cat Blood Pressure:

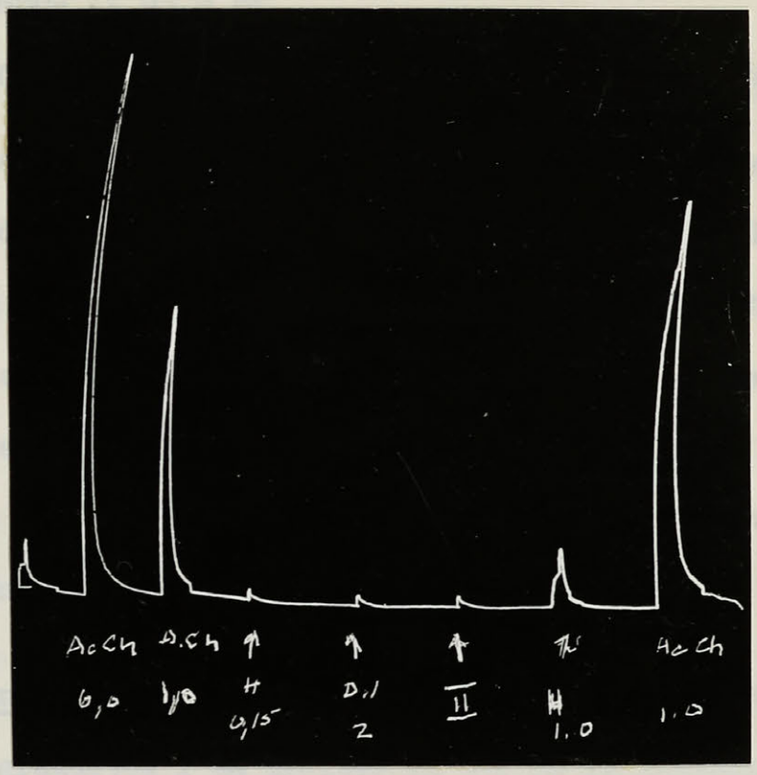
(a) The preparation: - The method for the assay of histamine has been described by Burn (1927). Cats weighing between 1.5 - 3.0 kg have been employed generally. They are anaesthetized with 'Dial' (Ciba) given intraperitoneally in a dose of 0.5 cc/kg body weight. The animal is usually ready after about twenty minutes. The blood pressure is obtained by cannulating the right carotid artery and using the usual mercury-manometer method for obtaining the blood

Figure 4



Example of Histamine Assay. 0.06, 0.08 etc. are Standard Solutions. $^{7}/_{100}$, $^{6}/_{100}$ are unknown Extracts.

Figure 5



Showing Paralysing Effect of a Large Dose of Histamine. The Gut Responds to Acetyl-Choline (AcCh) but does not Respond to Histamine Standards (H) or Tissue Extracts.

pressure tracing. Occasionally the femoral artery has been employed instead of the carotid.

The fluid in the blood pressure system contains equal parts of a 3.5% sodium citrate solution and a second solution which has the following composition:

$NaHCO_3$ - 93.0 gm.

NaC_2CO_3 - 143.0 gm. made up to 2000 cc with distilled water.

The injections of histamine are given intravenously. A small glass cannula is inserted into the femoral vein and it is connected to a 20 cc glass syringe containing normal saline by small rubber tubing about eight inches in length. A hypodermic needle is inserted into the lumen of this tube about two inches from the cannula and the tube is kept closed by an artery forceps just above the cannula between it and the hypodermic needle. All air bubbles must be removed from the system before an injection may be made.

(b) Dosage and assay of histamine: - To make an injection, the solution is measured out in a graduated 1 cc syringe of the O. T. variety. The syringe is inserted into the hypodermic needle, and after releasing the artery forceps, its contents are injected. All injections are washed in by 1 cc of ~~sodium~~ SALINE from the large syringe and the tube is again clamped. When the blood pressure has been recorded, the kymograph should be stopped in order to facilitate comparison with the next response. Injections can be made every three minutes.

Usually two standard histamine solutions are employed one having a histamine content of 1 y/cc and the other of 5 y/cc. Any strength of histamine solution may be used, but for accuracy the unknown should be diluted until it compares with a histamine solution of 0.3 to 1.0 y/cc.

If the presence of choline or acetyl-choline is suspected in an extract, the animal should be atropinized by giving a total dose of 1-2 mg of atropine sulphate. This takes about thirty minutes to exert its effect.

Examples of this type of assay are shown later (see Figs. 7 and 8.).

"Experimental Data."

The various experiments which are about to be described have been carried out on rats of the 'hooded' strain from the same colony. The animals were fed on a standard Purina diet.

In order to obtain the tissues or blood, the animals were first anaesthetized with ether, the abdomen was opened and blood was removed from the inferior vena cava and the amount was noted. Tissues were then removed and placed directly into a previously weighed beaker containing 10% HCl. The stomach or small intestine was always opened and washed clean. The excess moisture was then removed by placing the tissue on thick filter paper, and the tissue was then placed into the bottles containing the hydrochloric acid.

All values for histamine given in the experimental data are expressed as histamine base in gamma per gram of tissue or gamma per cc of blood.

A. Studies on the Intact Animal:

(1) The histamine content of the blood and tissues of the normal rat:

Experiments were carried out in order to determine the histamine content of the blood and tissues of normal animals. The results which are herein presented are taken in part from the controls of other experiments. Animals weighing from 150 - 180 grams were used, and observations were made on the histamine content of the blood, lungs, liver, muscle, stomach, small intestine and kidneys. The results are given in Table IV and in Figure 6.

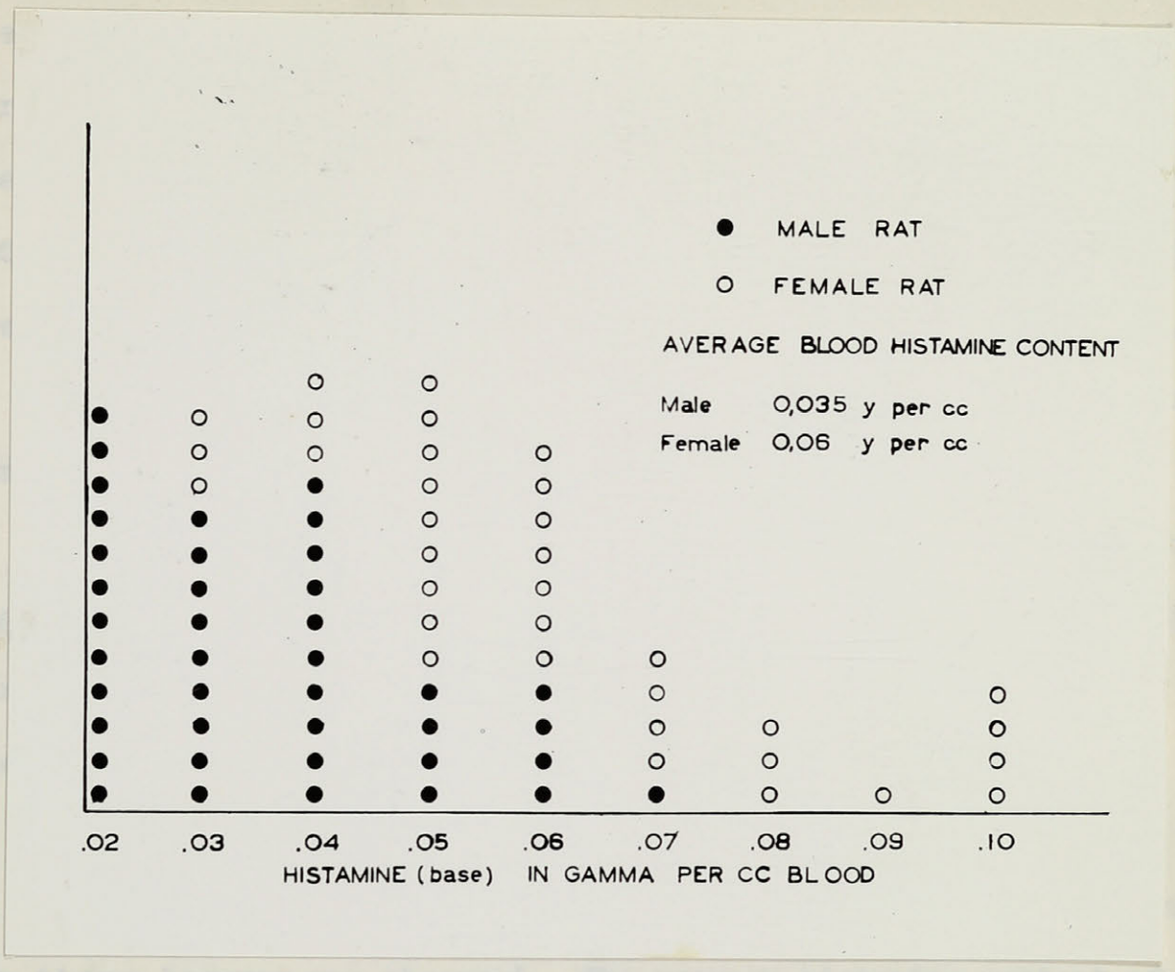
The histamine content of lung tissue was found to average 8.1 y/gm for females and 9.0 y/gm for males, with variations of from 6.2 to 13.2 in the females and from 5.5 to 16.6 in the males. These figures do not agree with those given by Raxlen (1930) who found values of 2 - 3 y/gm for the rat.

TABLE IV.

The Histamine Content of some Tissues of the Intact Rat

KIDNEY	LUNG		LIVER	MUSCLE		STOMACH		INTESTINE
Female	Male	Female	Female	Male	Female	Male	Female	Female
0.3	16.6	8.5	1.3	4.2	4.9	9.6	9.2	3.09
0.39	8.5	8.0	1.3	8.6	5.8		15.6	4.48
0.35	5.8	7.0	1.2	8.9			15.0	4.80
0.08	6.5	6.2	1.8	5.9			13.3	
0.35	9.1	10.7	1.8	5.2			15.0	
0.5	5.5	10.8		5.9			12.6	
0.6		7.4		5.1			14.8	
0.54		12.4		5.5			20.0	
0.43		7.8		4.0			8.0	
0.23		7.3					10.1	
0.6		8.5					10.6	
0.9		8.6						
0.6		13.2						
0.6		9.2						
0.9		6.8						
0.16								
1.6								
0.32								
Average values:								
0.54	9.0	8.1	1.4	5.9	5.3		13.2	4.12

Figure 6



Showing the Histamine Content of the Blood of the Male and Female Rat.

(2) Studies on the sex difference of the histamine content of the blood of the rat:

On examining the results obtained for the histamine content of whole blood, it soon became evident that there was a difference in the concentration of histamine in the blood of male as compared with female animals (Rose 1938). The average blood histamine of 40 male rats was found to be 0.035 y/cc whereas that of 34 female rats was found to be 0.06 y/cc (Fig. 6). These were all adult animals varying from 150 to 220 grams in weight.

Various procedures were performed in an attempt to determine the underlying mechanism for this difference.

(a) Ovariectomy: Eighteen adult female rats averaging about 180 grams in weight were divided into two groups of nine. One served as controls. The other group were ovariectomized. After one month the histamine content of the blood of both group was determined.

No change in the histamine content of the blood was noted, nor in that of the kidney. An increase in the histamine content of both lung and liver tissue however was observed. The average histamine content of lung rose to 19.8 y/gm as compares with 8.2 y/gm in the normal, that of the liver rose to 3.6 y/gm as compared to 1.4 y/gm in the normal. The results are shown in Table V.

TABLE V.

Effect of Ovariectomy upon Histamine Content of the Blood and Tissues of the Female Rat.

BLOOD		LUNG		LIVER		KIDNEY	
Control	Castrate	Control	Castrate	Control	Castrate	Control	Castrate
0.08	0.04	8.5	21.5	1.2	4.6	0.5	0.72
0.08	0.08	7.0	15.5	1.3	4.7	0.6	0.35
0.06	0.06	10.7	19.7	1.3	0.9	0.2	0.00
0.05	0.10	7.4	20.4	1.2	3.5	0.2	0.10
0.03	0.11	8.3	19.3	1.8	3.9		
0.05	0.09	7.3	22.4	1.8	4.2		
0.10	0.09						
0.07	0.09						
0.11	0.08						
Average:							
0.07	0.082	8.2	19.8	1.4	3.63	0.36	0.29

(b) Effect of castration: Thirty adult male rats weighing 180 grams on the average were divided into two groups. Ten animals served as controls. The remaining twenty were castrated and at intervals of one to two months after castration the histamine content of the blood was determined.

It will be seen that the average histamine content of the blood definitely increased, the histamine content of the blood of the control animals being 0.038 y/cc, whereas that of the castrates was 0.077/cc. (see Table VI).

On the examination of the figures obtained for the blood histamine of the castrate animals, it will be noted that although the average histamine content is higher after castration, the results are somewhat variable.

TABLE VI.

The Effect of Castration on the Histamine Content of the Blood of the Male Rat.

BLOOD HISTAMINE	
Controls	Castrates
0.04	0.06
0.03	0.05
0.035	0.05
0.04	0.04
0.03	0.06
0.04	0.05
0.05	0.04
0.04	0.035
0.045	0.05
0.035	0.24
	0.16
	0.07
	0.16
	0.08
	0.06
	0.12
	0.05
	0.08
	0.06
	0.06
	0.05
Average 0.038	Average 0.077

Discussion:

Previous observations have indicated that there is a sex difference in the histamine content of the gonads. In Table I (see p.10) it will have been noted that the ovary contains approximately ten times the amount of histamine which is found normally in the testis (Gaddum and Dale 1936). The relationship of the gonads to histamine metabolism is not understood, although there are several isolated findings which seem to indicate that some interrelationship exists. For example, Marcou et al (1938) found that the histamine content of the blood of pregnant women diminished at the time of labour. They also noted that there was a marked increase in the blood histaminase at this time. Danforth and Gorham (1937) and Danforth (1939) have shown there is some relationship to the amount of histaminase found in the placenta and the efficiency of uterine contractions. They do not believe however that the evidence is sufficiently definite to warrant such a conclusion. It is also interesting to note that Ungar and Dubois (1937) found a histamine-like substance in the urine of pregnant women. They examined 107 specimens and found histamine in 72 cases. On the other hand the urine of non-pregnant women and normal man did not contain histamine (Ungar and Pocoulé 1937).

B. The Effect of Adrenalectomy on the Histamine Content of the Tissues of the Rat.

The relationship of the adrenal glands to the metabolism of histamine has been discussed in a previous section (p.114). It was decided to study the effect of adrenalectomy upon the histamine content of the tissues of the rat.

Rats weighing between 150 and 180 gms were used. Under ether anaesthesia, the adrenal glands were removed, through two lateral incisions. Care was exercised not to rupture the cortex so as to prevent regeneration of cortical tissue. The animals were then placed on the usual Purina diet and were given 0.85% sodium chloride to drink instead of water. This diet plus the normal saline to drink maintains these animals in a healthy condition for as long as several months. Since it has been established that sensitivity to histamine is increased only about five to six days after removal of the adrenal glands (Marmorston-Gottesman and Perla 1931) these animals were kept for seven days after adrenalectomy. They were then killed and the tissues were removed and examined for their histamine content.

TABLE VII

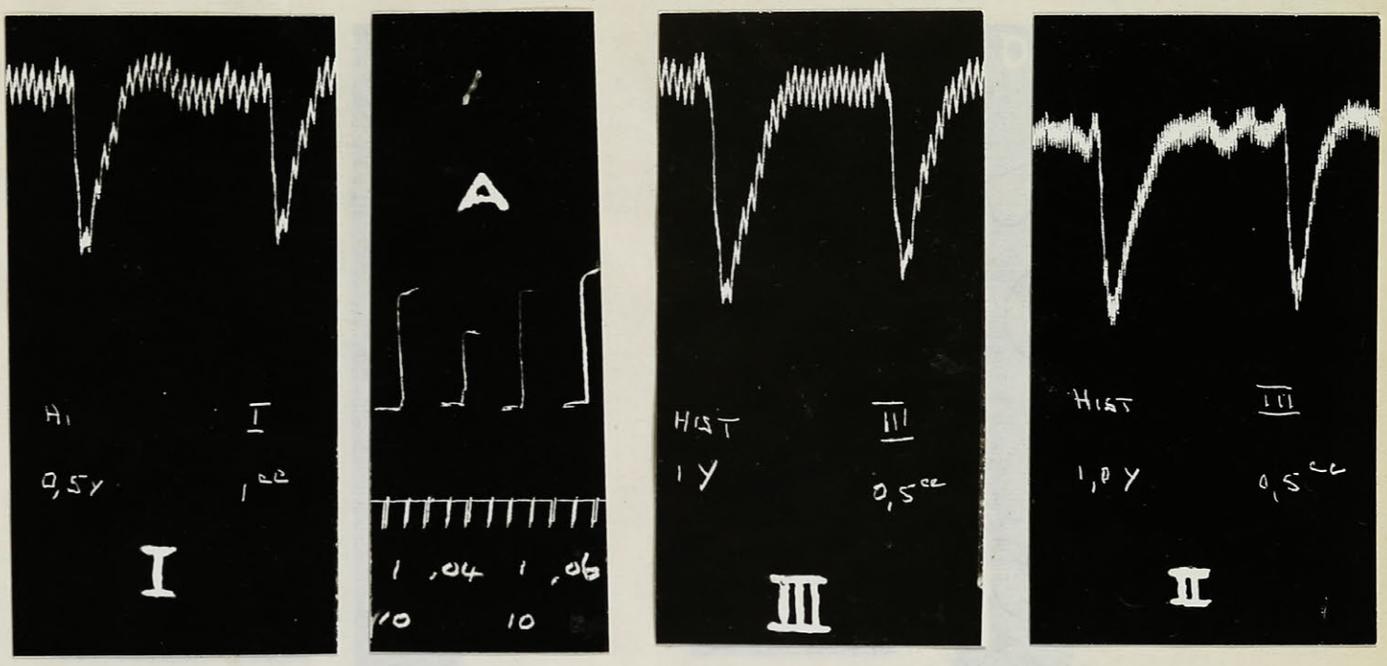
The Effect of Adrenalectomy on the Histamine Content of the Tissues of the Rat.

MUSCLE		LUNG		STOMACH		SMALL INTESTINE	
Normal	Adrenal-ectomized	Normal	Adrenal-ectomized	Normal	Adrenal-ectomized	Normal	Adrenal-ectomized
4.2	5.8	8.5	9.5	9.2	26.4	3.09	10.7
8.6	4.7	8.0	11.3	15.6	17.3	4.48	11.6
8.9	5.9	7.0	6.5	15.0	19.5	4.80	6.6
5.9	6.3	6.2	9.5	13.3	20.0		8.0
5.2	5.4	10.7	5.8	15.0	24.0		10.0
5.9	5.8	10.8	12.7	12.6	31.0		
5.1	5.9	7.4	12.5	14.8	30.0		
5.5	4.6	12.4	11.5	20.0	21.0		
4.0	7.6	7.8		8.0	20.0		
		7.3		10.1	27.0		
		8.5		10.6	25.0		
		8.6			31.8		
		13.2			44.4		
		9.2			44.4		
		6.8					
Averages:							
5.9	5.7	8.1	10.0	13.1	27.2	4.12	9.35

On examination of the results (see Table VII) it will be seen that there is a marked increase in the histamine content of both stomach and small intestine. The increase in the stomach being 100% and that in the small intestine 126%. A much smaller increase namely 23% is observed in the histamine content of the lung and no change occurs in that of the muscle. Examples of the assay of extracts of both stomach and small intestine may be seen in Figures 7 and 8.

Since it is known that adrenalectomy will affect the weights of various organs, the average weights of the lung and stomach were estimated in a series of normal and in a series of adrenalectomized animals, maintained on a Purina diet and 0.9% NaCl, the tissues of which were removed seven to ten days after removal of the adrenal glands. The results are given in Table VIII .

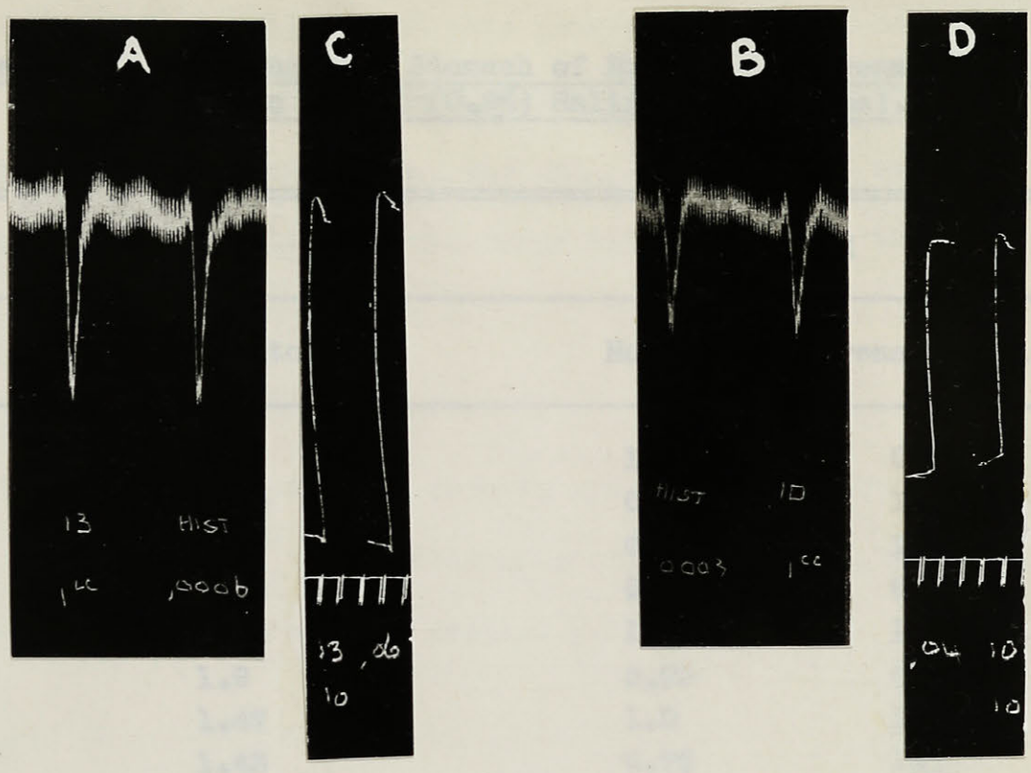
Figure 7



Example of Assay of Extracts of Stomach Normal and Adrenalectomized Animals on Cat Blood Pressure and Guinea Pig Gut.

Ext. No. 1 (normal stomach) I and A
Ext. " 3 Adrenalectomized Stomach
before Atropine III
after Atropine II
Ext. " 10 Adrenalectomized Stomach B.

Figure 8



Example of Assay of Extract of Intestine from Normal and Adrenalectomized Animals.on Cat Blood Pressure and Guinea Pig Gut.

A and C = Ext. No. 13 (adrenalectomized
B and D = " " 10 (normal)

TABLE VIII.

Average Wet Weight of Lungs and Stomach of Normal and Adrenalectomized Rats Maintained on Normal (0.9%) Saline. (in grams).

LUNG		STOMACH	
Normal	Adrenalectomized	Normal	Adrenalectomized
1.66	1.0	1.89	0.85
0.9	1.39	0.86	1.3
1.1	1.37	0.88	1.1
1.19	1.58	0.94	0.9
1.0	1.45	1.19	1.12
1.43	1.9	0.95	0.98
1.14	1.47	1.0	1.0
1.25	1.43	0.75	1.2
1.23	1.8	0.73	0.9
1.0	1.3	0.93	1.0
0.88	1.35	0.85	0.94
1.3	1.4	0.99	1.1
1.3	1.3	0.85	0.9
1.2	1.35	0.83	0.9
1.4	1.32	1.00	
1.2	1.61		
1.8	1.57		
1.13	1.5		
1.2	1.3		
1.3	1.3		
	1.7		
	1.5		
	1.55		
	1.3		
Average Weights:			
1.23	1.44	0.98	1.01

It will be seen that there is an increase of 8% in the weight of the lungs after adrenalectomy, while an insignificant change takes place in weight of the stomach. If this increase in weight were due to water it would tend to lower the histamine concentration providing that the total histamine content of the lungs was not altered. This would make the increase in the histamine content of the lungs slightly higher than 23% after adrenalectomy.

Discussion:

The discussion of these results and of two subsequent series of experiments which also deal with the relationship of the adrenal gland to histamine will be found together in a later section (see p.180).

C. The Distribution and Rate of Disappearance of Histamine Injected Intravenously in the Rat.:

(1) Intact animal:

Although observations made by previous workers have indicated that histamine may be inactivated rapidly in the organism (Oehme 1913; Dale and Laidlaw 1910; Dragstedt and Mead 1935) the actual rate of disappearance and the fate of the injected histamine had not been determined. Dragstedt and Mead (1935) noted that if a dose of 0.5 mg of histamine per kg was injected intravenously into a dog, histamine was detectable in the blood at two minutes after the injection but not at five. Traces were observed in the blood at thirty to forty minutes after the injection of 3.0 to 4.0 mg/kg.

It was therefore thought that it would be of interest to study the fate of intravenously injected histamine.

The same method of injection was used in this group of experiments, the only difference being in the size of the dose. Solutions of histamine HCl were made up in 0.85% NaCl. The animals were first anaesthetized, and the jugular vein was exposed on one side. The solution of histamine was then rapidly injected intravenously, the wound closed and the animal allowed to recover. The volume of the injected fluid at no time exceeded 2 cc. After a definite period the animal was again anaesthetized, the abdomen opened and a sample of blood removed from the inferior vena cava. The tissues were then removed as previously described.

(a) Rate of disappearance of large intravenous doses of histamine from the blood: - In a group of eleven adult male rats weighing about 250 grams, an intravenous injection of 96 y of histamine per gram body weight was given. The total amount injected would then be 24,000 y (40 mgm of histamine HCl, the salt used throughout the experiments).

The animals were killed at 3, 5, 10, 15, 30 minutes, 1, 2, 3, 4, 5

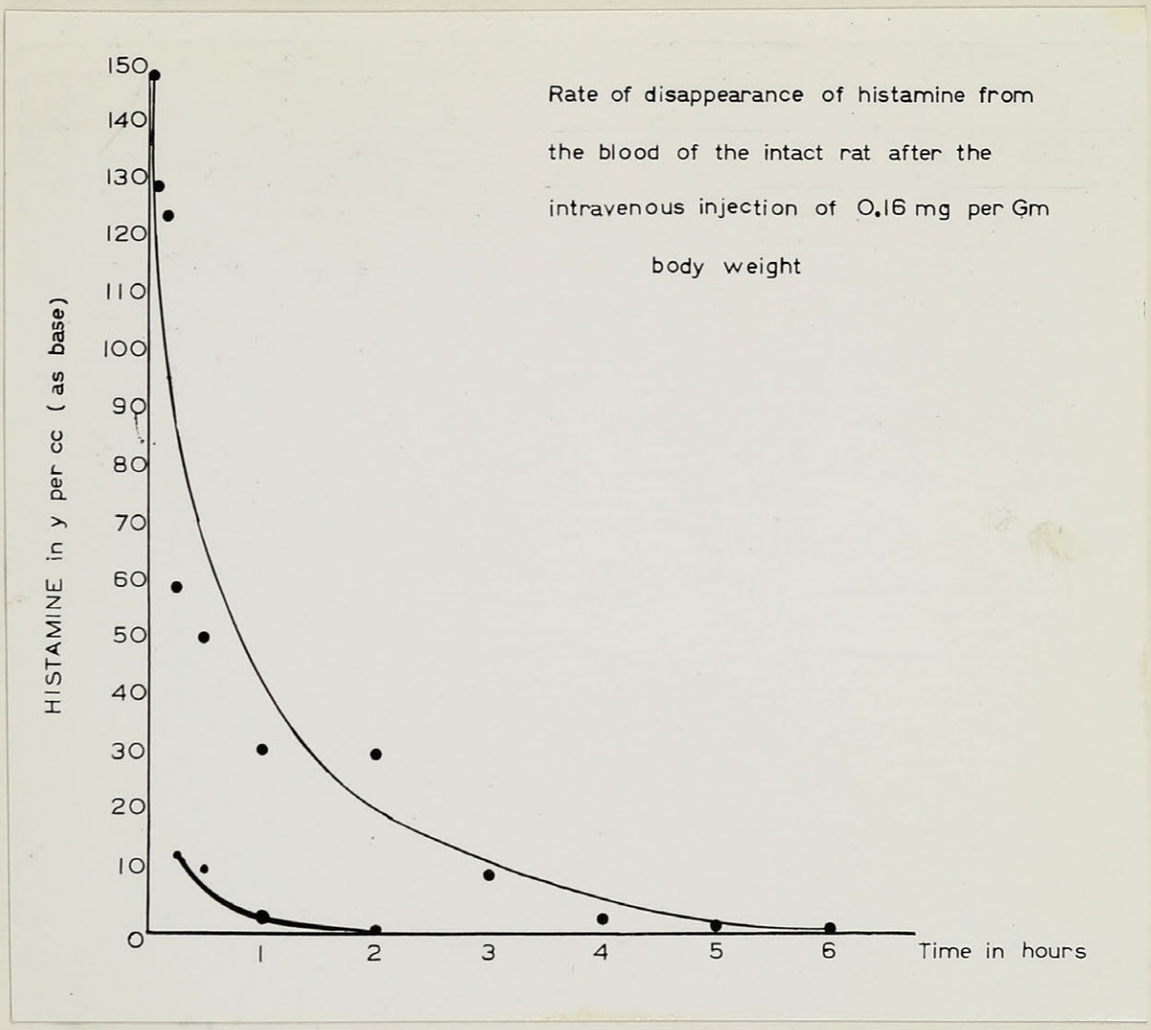
and 6 hours after the injection had been given. The resulting curve is seen in Figure 9. It will be seen that the blood histamine content is 150 y/cc at three minutes after the injection. Even assuming that no decrease of blood volume took place and that such an animal has a blood volume of 12 cc the total histamine content of the blood would be 1800 y or 7.5% of the total amount originally injected. If a decrease of blood volume occurred as early as this even less is recovered. The blood histamine decreases rapidly as will be seen. At the end of six hours however, a small amount may still be detected the value being 2 y/cc, (normal blood histamine 0.035 y/cc).

(b) Distribution and Rate of disappearance of intravenously injected histamine from the blood and tissues of the rat: - From the results of the foregoing experiment it is evident that histamine disappears from the blood of the rat when it is injected intravenously.

In order to determine further the fate of this intravenously injected histamine, experiments were carried out on adult male and female rats weighing from 180 - 220 grams, in which the tissue histamine content was estimated as well as that of the blood. Since it was desired to study the effects of adrenalectomy and since it is well known that adrenalectomized animals are less resistant to histamine than intact animals a smaller dose namely 24 y of histamine per gram body weight was therefore used so that results might be compared in the normal and adrenalectomized animal. In such an animal the average dose of histamine injected was about 4800 y (8.0 mgm histamine HCl).

The histamine was injected in the same manner as described in the foregoing experiment, but in this series the animals were killed fifteen, thirty minutes, one, two and three hours after the injection. The tissues studied were blood, lung, liver, kidney and lymph glands, stomach, small intestine, spleen and appendix. The results are expressed in Table IX and Fig. 10. The results expressed in these tables for the blood, lung, liver and

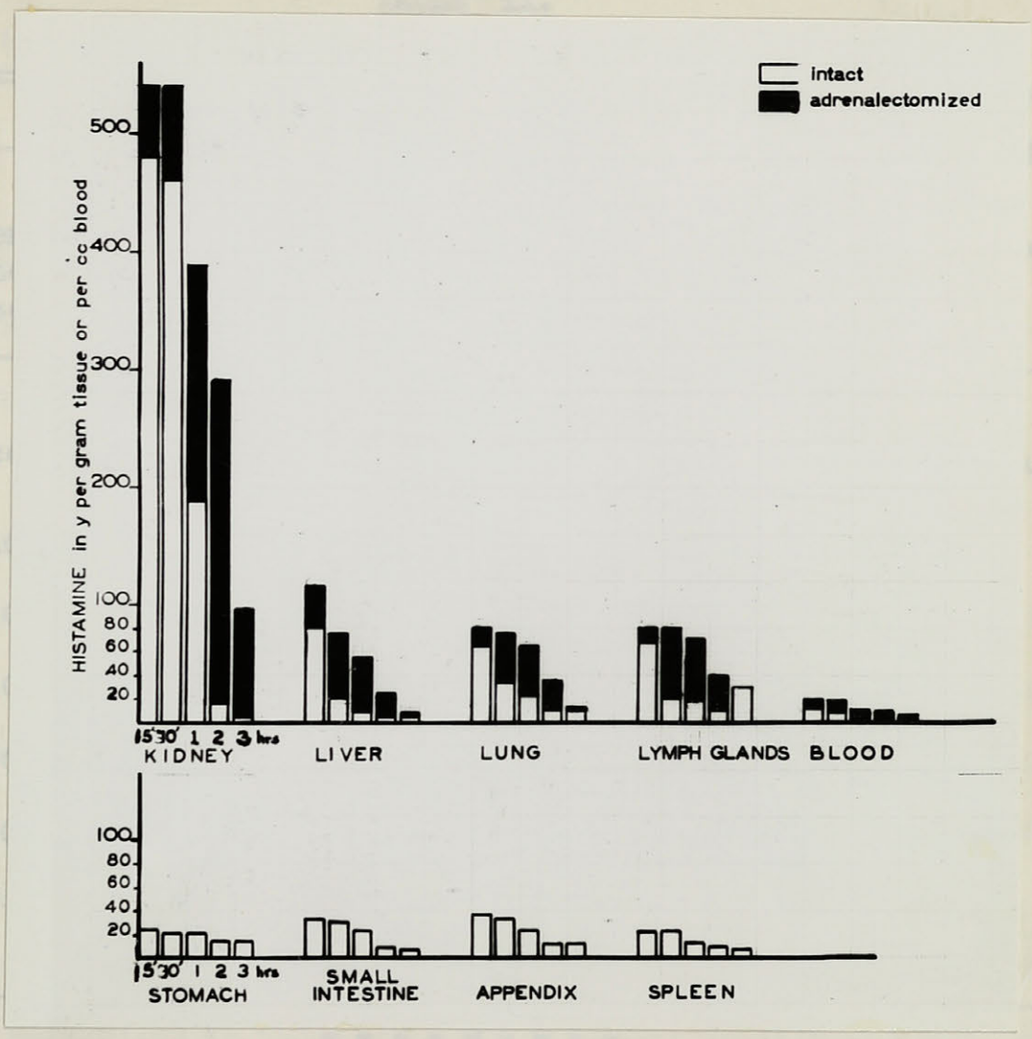
Figure 9



The Small Curve is that Obtained after an Injection of One Quarter the Dose, namely 0.04 mg per gm.

kidney of normal and adrenalectomized animals represent the average of six experiments in most cases. Those of the remaining tissues are averages of three experiments.

Figure 10



Distribution and Rate of Disappearance of Histamine from the Tissues of the Rat following the Intravenous Injection of 24 gamma of Histamine (Base) per gram Body weight. The Height of the White Columns denotes the Amount found in the Tissues of the Intact Animal. The Height of the black Columns denotes the Amount in gamma per gram found in the Tissues of the Adrenal-ectomized Animal at the same Time Period. The abcissa represents time after the Injection and is the same for each Tissue.

TABLE IX.

me	Blood		Kidney		Lung		Liver		Lymph Gland	
	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized
urs	y/cc	y/cc	y/gram	y/gram	y/gram	y/gram	y/gram	y/gram	y/gram	y/gram
/4	13.2	20.0	480	540	64	80	79	115	68	80
/2	9.7	18.0	460	540	34	75	21	74	20	80
1	3.0	12.0	188	388	22	65	8	53	18	70
2	0.5	10.0	17	290	10	35	4	25	10	40
3	0.2	7.0	4.0	95	10	12	4	7	30	30

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It will be observed from these results that even at fifteen minutes, the distribution of the histamine is markedly in favour of the tissues as compares with the blood. The blood value was 14.0 y per cc or about 280 times the normal. The histamine content of the lung was 64 y per gram whereas the kidney histamine content was far in excess of any of the other tissues studied, being 480 y /gram or about 1600 times its normal value.

The histamine content of the other tissues, namely stomach, small intestine, appendix and spleen was relatively only slightly raised, the increase never being greater than 20 times the normal amount fifteen minutes after the injection.

A consideration of the rate of disappearance of histamine from the tissues reveals that during the fifteen to thirty minute period, the concentration in the kidney decreases slightly or not at all. In the thirty minute to one hour period, however, a marked decrease in the histamine content of this organ takes place, namely from 460 y per gram to 188 y per gram (Fig.11). At the end of three hours, all values of the tissues approach normal with the exception of the blood and kidney which are still raised to some ten times the normal. Although the histamine content of the lymph glands is also raised, marked variations in the results in different experiments were noted.

If the histamine content of all the tissues studied is totalled at the fifteen minute period, 1540 y (32%) of the injected histamine is accounted for of which 720 y (15%) are in the kidney (see Table X).

Figure 11.

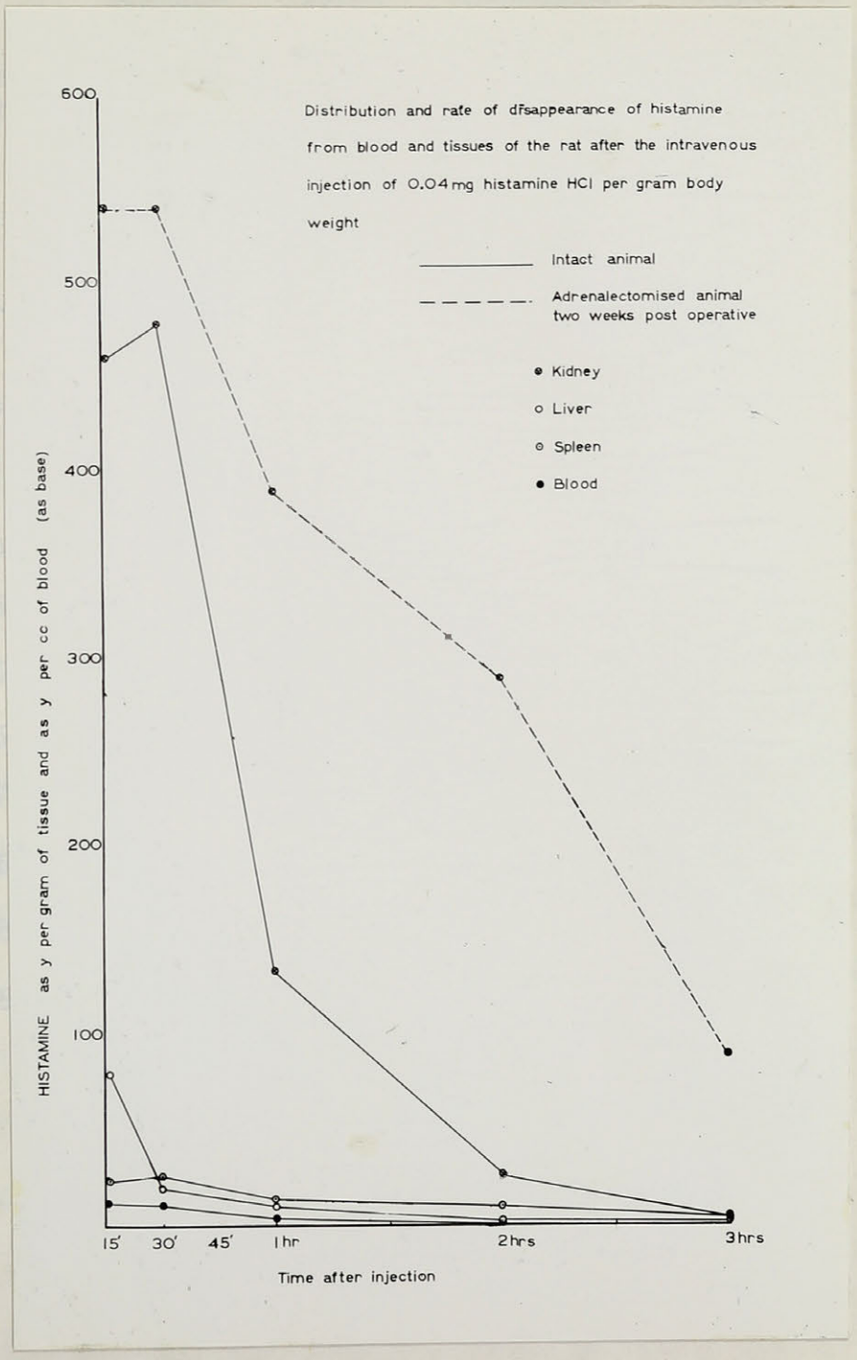
Distribution and rate of disappearance of histamine from blood and tissues of the rat after the intravenous injection of 0.04 mg histamine HCl per gram body weight
Intact animal
Adrenalectomised animal two weeks post operative
Kidney
Liver
Spleen
Blood
HISTAMINE as y per gram of tissue and as y per cc of blood (as base)
600
500
400
300
200
100
15'
30'
45'
1 hr
2 hrs
3 hrs
Time after injection

TABLE X.

Tissue	Average Weight	Average Histamine Concentration Normal	Total Histamine Concentration Normal	Histamine Concentration 15 Minutes after Injection	Total Histamine Concentration 15 Min. after Inj.	Distribution of recovered Histamine 15 Min. after Inj.	Percentage recovered at 15 Min. of total amount of Histamine injected
	grams	y/gram or/cc	y	y/gram or/cc	y	per cent	
›od	10 cc	0.05	0.5	14.0	140	9.1	2.91
ıg	1.5	4.0-10.0	6.0-15.0	65.0	97	6.3	2.0
‹er	4.5	2.0- 4.0	9.0-18.0	80.0	360	23.4	7.5
lney	1.5	0.0- 0.4	0.6	480.0	720.0	46.7	15.0
.een	0.7	2.0- 5.0	3.5	25.0	17.5	1.15	0.36
ıll intestine	4.5	5.0-6.0	27.0	30.0	135	8.75	2.81
›endix	0.8	10.0	8.0	38.8	31	2.1	0.66
›mach	1.8	9.0	16.2	22.0	39.6	2.5	0.82

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(2) Adrenalectomized animals:

Experiments were carried out on adrenalectomized animals under the same conditions as described in the foregoing experiments. The animals were from the same colony and of the same approximate weight. Adrenalectomy was carried out as previously described, and the animals were maintained in good health, being fed on a Purina diet, and given 0.85% saline to drink. Only blood, lung, liver, kidneys and lymph glands were studied. The same dose of histamine, namely 24 y/gm body weight was injected, and the blood and tissues were removed at the same time intervals.

These animals will tolerate such a dose of histamine, although not as well as the intact animal.

In preliminary experiments, animals were used five days after the adrenalectomy and in this case, no definite change in the distribution of the histamine or in the rate of its disappearance could be noted as compared with the normal. Subsequent experiments were performed on animals which were adrenalectomized at least ten days prior to the experiment. The results are shown in Table IX and Figure 10. It will be observed that at fifteen minutes after the injection, the distribution is in general the same as that for the intact animal. The blood histamine content however is higher even at fifteen minutes, it being 20.0 y per cc as compared with that of the intact animal at this time which is 14.0 y/cc.

In the subsequent time periods however, a marked retardation in the rate of disappearance of histamine from the blood and all the tissues is observed. This retardation is definite at the thirty minute period and is most marked at the first and second hours. Of all the tissues studied, the kidney is most affected. At the three hour period, all of the tissue values are approaching normal with the exception of that of the kidney which is still 200 times the resting normal amount, and that of the blood which is 100 times the normal amount. The discussion of these results will be found on page 180

(3) The effect of the administration of cortin and of desoxycorticosterone acetate on the rate of disappearance of intravenously injected histamine in the adrenalectomized rat:

It has been shown by various observers that the increased sensitivity to histamine which occurs following adrenalectomy in the rat may be restored to normal by the administration of adreno cortical hormone preparations. (Marmorston-Gottesman and Perla 1931; Wyman 1928; Ingle 1936).

It seemed of interest therefore to determine the dose of cortin and of a synthetic compound namely desoxycorticosterone acetate which would be required to restore the ability of the adrenalectomized rat to inactivate histamine.

Rats, divided into groups of six were first adrenalectomized and maintained on Purina and 0.85% saline. On the seventh day treatment with either cortin (Wilson) or desoxycorticosterone acetate was begun, and continued for four days, the doses being varied.

The preparation of cortin used was that prepared by the Wilson Laboratories, of which 0.5 cc daily injected subcutaneously is regarded as a maintenance dose for the adrenalectomized rat. The desoxycorticosterone acetate was used in an oily preparation 1 cc of which is equal to 5 mgm of the acetate. It is injected intramuscularly.

In all, six groups of animals were treated and in each case the last injection of cortin or desoxycorticosterone acetate was given 12 hours before the injection of histamine.

As in the two series of experiments previously described a dose of 24 y of histamine per gram body weight was injected intravenously. Since it has been shown that the decrease in the ability of an adrenalectomized animal to destroy histamine is most apparent at the first and second hour after an intravenous injection of histamine has been made, the animals were killed at periods of one, two and three hours. The tissues removed for study at these times were blood, kidney and liver. - The results are given in Table XI.

TABLE XI.

Effect of Varying Doses of Cortin and of Desoxycorticosterone Acetate on the Rate of Disappearance of Intravenously Injected Histamine in Adrenalectomized Animals.

DATA	BLOOD			LIVER			KIDNEY		
	1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour
rmal animals	3.0	0.5	0.2	8.0	4.0	4.0	188	17	4.0
renalectomized animals	12.0	10.0	7.0	53	25	7.0	388	290	95
renalectomized animals rtin 0.5 cc lly for 4 days	11.5	8.8	2.9	34.0	14.7	9.9	224	206	81.0
renalectomized animals rtin 2 cc daily for 2 days and 2 cc x 2 for 2 days	8.4	3.5	1.7	25.7	6.4	3.3	265	124	37.7
renalectomized animals ortin 2 cc x 2 for 3 days and cc x 4 for 1 day	1.8	1.0	0.45	15.1	3.9	2.5	81	13.8	9.7
renalectomized animals esoxycortico-sterone 5 mg x 2 for 3 days and 0 mg x 2 for 1 day	6.5	3.2	0.7	20.0	6.4	3.1	236	53	11.0

It will be observed that if a maintenance dose of cortin, namely 0.5 cc daily, is given subcutaneously to adrenalectomized animals for four days, little or no increase in the ability of the animals to inactivate histamine is observed. It was found that a dose 2 cc twice daily for three days followed by four daily injections of 2 cc for a fourth day was able to restore the ability of the animals to inactivate histamine to within normal limits.

When desoxycorticosterone is used, it will be seen that 5 mgms. daily for three days following by 10 mgms. for a fourth day restores the ability of adrenalectomized animals to inactivate histamine to within normal limits.

During the course of the experiments it seemed however that the animals treated with adequate doses of cortin were in better condition as judged by their general reaction to the injection of histamine and by the amount of available blood, than those treated with the synthetic product.

This may be due to the fact that the cortin extract contains more than one principle of the adrenal cortex, whereas the synthetic preparation is a concentrate of one factor only.

Discussion:

The discussion of these results will be found in conjunction with the other experiments dealing with the relationship of the adrenal gland to histamine metabolism (see page 180).

"Studies on Histaminase."

In a previous section (p.20) the various mechanisms by which histamine may be destroyed in the animal organism were discussed. Amongst these, histaminase, an enzyme system which was described by Best (1929) and Best and McHenry (1930) is the only one which is well understood. It was pointed out that the enzyme acted best at a pH of 7.2, and at a temperature of 37°C, and that it inactivated histamine by a process of deamination in which a molecule of ammonia was liberated for each molecule of histamine inactivated. The greatest source of this substance is found in the kidney tissue of most mammals. It was shown however by McHenry and Gavin (1932b) that the kidneys of the rat and guinea pig did not contain any histaminase.

In order to determine what the mechanism of the destruction or inactivation of histamine might be in the rat, it was decided to study the histaminase content of the various tissues in this animal.

(1) First Method:

Because of the available amounts of tissue and for the sake of convenience the following simple method was adopted.

The animals were first anaesthetized; the organs were removed and washed. The stomach and intestine were always opened and thoroughly cleaned. These tissues were then finely ground through a small tissue grinder. Varying amounts of the finely minced tissue were placed in small wide-mouthed beakers containing 20 cc of phosphate buffer at pH 7.2 and 1 cc of a solution of histamine (1.0 mgm of histamine (base) per cc). Toluol was finally added and the whole placed in an incubator at 37°C for sixteen hours. After incubation the specimens were brought to the boiling point and after cooling they were made up to a volume of 50 cc with normal saline and assayed

directly. The amount of histamine present indicated how much had been destroyed by the tissue. Several specimens were put through the method for the extraction of histamine from tissues as described in the section on methods (see p.126). Results of the histamine assay on both types of extract were always in agreement. In these as in other experiments the figures for histamine are expressed in gamma. (see Table XII).

Assays were made on the guinea pig ileum preparation. The following tissues were studied: lung, liver, small intestine, kidney and blood.

TABLE XII.

Histamine Content of Solutions after Incubation with Rat Lung Tissue.

Comparison of Results obtained by (1) Direct Assay; (2) Assay after Extraction.

Weight of Tissue	Direct Assay	After Extraction
0.5 gm	700	
0.5 "	650	
0.5 "	750	
0.5 "		700
0.5 "		680
0.5 "		720

In the first series of experiments it was observed that, although toluol was always added to the contents of the beakers, contamination of the specimens by bacterial growth was a frequent complication. This occurred mostly in the specimens containing intestine and liver. It also occasionally occurred in the specimens containing lung tissue. The presence of bacterial contamination was easily noted by the putrid odour of such specimens.

Although contaminated specimens were generally discarded, some were assayed for their histamine content. The results obtained were extremely variable, a marked increase over the initial amount of added histamine being found in some, whereas a marked decrease was found in others.

Discarding the contaminated specimens, it was found that of all the tissues studied, namely lung, liver, kidney, small intestine, spleen and blood only two were capable of destroying histamine. These were namely lung and small intestine. (see Table XIII).

TABLE XIII

Ability of Various Tissues of the Rat to Inactivate Histamine.

(First Method).

Exp. No.	Lung in grams	Histamine destroyed	Intestine in grams	Histamine destroyed	Blood	Kidney	Spleen	Liver	Control
42	1.4	450	6.5	350	0	0		0	1000
47	2.3	430	5.8	425		0	0		
60	1.3	550	7.6	700		700		0	5 controls all inactive
64	1.4	300	4.0	0	0	0	0		0

It was noted at this point that control specimens, i. e., Buffer solution containing 1.0 mgm of histamine to which toluol had been added, were also frequently inactivated.

It was then decided to study the effects of various procedures on the histaminase content of the lung.

A series of twenty-one experiments was carried out in which varying amounts of lung tissue from adult male and female rats were incubated with 1.0 mgm of histamine as described above. These included the effects of pretreatment of animals with vitamin C, thyroxin and the effects of hypophysectomy. Examples of the results all of which were negative may be seen in Table XIV.

TABLE XIV.

Histaminase Content of Normal Rat Lung and of the Lung Tissue from Rats after Various Procedures.

(First Method).

Exp. No.	Remarks	Amount of Tissue	Histamine Destroyed
64	Normal lung	0.6	450
		1.4	300
		1.0	600
47	" "	2.3	430
		1.2	625
101	Animals received thyroid gr. 2 for 8 days	6.6	500
		0.9	600
		2.5	450
102	Thyroidectomized animals	0.8	500
		0.5	500
127	Animals treated with 50 mg of vit. C daily for seven days	1.59	600
	Control	1.4	625
106	Hypophysectomized animals	0.4	500
		0.5	600
		0.7	500
	Controls	0.7	700
		0.95	700
		0.7	700

Because of the fact that most of the results obtained in the experiments using this first method are not acceptable for reasons which will be pointed out later, some of the data has been left out.

A series of experiments on the effect of adrenalectomy on the histaminase content of rat lung tissue were also carried out.

Rats were adrenalectomized as previously described and maintained on normal (0.9%) saline. After eight to ten days a group of four adrenalectomized and two control animals were killed, the lung tissue was removed, and its histaminase content was determined. The lungs from the control animals were examined at the same time. Ten experiments were done in all. The results of six of these are shown in Table XV.

TABLE XV.

The Effect of Adrenalectomy on the Histaminase Content of the Lung of the Rat.

(First Method)

	NORMAL		ADRENALECTOMIZED	
Exp. No.	Grams of Tissue	Histamine Destroyed	Grams of Tissue	Histamine Destroyed
57	1.4	625	2.35	600
67	1.8	200	2.0	100
81	0.5	600	3.0	100
			2.3	200
86	0.5	750	2.0	420
			1.7	400
89	1.2	400	2.4	450
	1.2 heated	0	2.3	550
91	0.3	300	2.0	400

It was observed from these results that tissue from normal animals was capable of inactivating more histamine than that from adrenalectomized animals. Since the quantity of tissue from the control and adrenalectomized animals was varied, a strict comparison could not be made, but the figures seemed convincing if the amount of histamine destroyed were computed on a weight basis. For example in experiment 86, Table XV, the tissue from the control animal is capable of destroying 1500 y per gram whereas only 210 y/gram is destroyed by the tissue from adrenalectomized animals.

It was assumed however that greater amounts of tissue should be capable of inactivating proportionately larger amounts of histamine than smaller quantities of tissue if the mechanism were a true enzyme system. It was soon noticed however that this was not the case and that in fact the reverse was true (see Table XVI).

TABLE XVI.

Effect of Varying the Amount of Lung Tissue on the Ability of the Lung to Inactivate Histamine.

(First Method)

Exp. No.	Weight of Tissue	Histamine Destroyed
120	0.53	600
	0.66	600
	1.0	800
	1.6	600
126	0.5	450
	0.8	400
	1.0	550
	1.5	400
	1.6	500
130	0.15	640
	0.25	600
	0.5	400
	2.0	350
	2.1	350

Small amounts of tissue inactivated relatively large amounts of histamine and as more lung tissue was added, less histamine was destroyed.

In order to substantiate this, use was made of a standard commercial histaminase preparation supplied by the Winthrop Chemical Company. This preparation (Torantil) made from dog intestinal mucosa is supplied in ampoules containing approximately 15 mgms, each of which is capable of destroying 1 mgm of histamine in twenty-four hours. Two experiments were done. In the first the contents of one ampoule was incubated with increasing amounts of histamine. In the second, the amount of histamine was kept constant, but the quantity of the histaminase was varied. The results are shown in Table XVII.

TABLE XVII

Potency of Torantil (T. 360), a Histaminase Preparation.

Exp. No.	Amount of Powder in mgm	Histamine Incubated in y	Histamine Destroyed in y
	(one ampoule)		
123	± 15	1000	440
	± 15	2000	720
	± 15	3000	1000
	± 15	4000	1200
	± 15	5000	2600
131	10	1000	300
	15	1000	520
	20	1000	650
	30	1000	890

It will be observed that the destruction of histamine takes place in a direct ratio to the amount of histaminase present, that is, the greater the amount of histaminase, the more histamine destroyed. Further the destruction of histamine by a fixed amount of histaminase increases in direct ratio to the concentration of histamine present.

On the basis of these experiments therefore, it was thought that the results obtained in the previous experiments on tissues might be explained by the presence of an inhibiting factor. That is if lung tissue contained a substance which might inhibit the action of histaminase, the greater the amount of tissue present, the less histamine destroyed, due to increase of the inhibiting factor. This would account in part then for these results.

A series of experiments was therefore carried out in which various types of extracts were prepared. In the first series, lung tissue was removed in the usual way. It was then ground up and placed in Buffer solution at 38°C for thirty minutes. This was then filtered or centrifuged. The filtrate (a) and residue(B) were then examined for their histaminase content by incubating with histamine in the usual way. The results are shown in Table XVIII. It will be seen that both residue and filtrate were capable of inactivating histamine, but not in a direct ratio to the amounts present (Exp. No. 133, Table XVIII).

TABLE XVIII

Activity of Various Histaminase Preparations.

(First Method).

Exp. No.	Weight of Residue in grams		Histamine Destroyed	Amount of Filtrate in cc	Histamine Destroyed
33 A	B	0.1	300	5.0	400
		0.2	400	6.0	200
ine		0.6	400		
racts		1.16	400		
33 B	B	0.5	300	1.0	0
ine		1.0	300	3.0	200
racts				6.0	200
35	B	0.5	800	1.0	0
				2.0	100
tone				4.0	200
racts				8.0	250
36		0.1	300	2.5	0
tone		0.2	500	5.0	200
racts		0.3	400		

The presence of an inhibiting factor was further looked for by the following method: Beakers containing Buffer solution and 1.0 mg of histamine and one ampoule of histaminase (Torantil) were incubated with and without the addition of either the residue (B) or the filtrate (A). Practically the same amounts of histamine were destroyed in all samples, indicating that an inhibiting factor could not be present.

A second series of extracts based on the method for the preparation of histaminase as described by Best and McHenry (1930) were prepared. Freshly removed lung tissue was finely ground up and extracted with 99% acetone for 10 hours. It was then filtered. The filtrate (A) was evaporated to dryness in vacuo. It was then taken up in a small amount of Buffer solution, and this was examined for the presence of an inhibitory factor as described above. None was found to be present.

The residue (B) was examined in two ways. Part was incubated directly with histamine and although it was found to be potent, relatively smaller amounts of histamine were destroyed in comparison to that generally inactivated by the original quantity of lung tissue from which it was derived. A second portion was examined for the presence of an inhibitory factor as described above but this was not found.

In other experiments, the residue (B) was placed in Buffer solution for 1 hour at 37°C. It was then filtered and the residue and filtrate were examined both for their histaminase content and the presence of an inhibitory factor. This latter substance was not found. The histaminase action will be seen by the results of two experiments, No. 135 and 136 in Table XVIII. It will be observed that whereas some of these extracts inactivated histamine in a direct ratio the majority of the results were still inconsistent. Because of the inconsistency of the results obtained using this first method it was felt that they could not be accepted.

(2) Second Method:

On the advice of Dr. E. W. McHenry it was decided to use special glassware kept only for histaminase experiments. The instruments used in the preparation of the tissue were all kept clean and sterile. A further change was also made in that small Erlenmeyer flasks were substituted for the wide-mouthed beakers, and finally a small amount of cotton wool was placed in the mouths of the flasks during the period of incubation.

Lung tissue was used, and the same procedure was followed as in the first experiments. It was now found that the amount of histamine inactivated was in direct ratio to the amount of tissue present.

(a) Histamine in Normal Rat Tissues:

Using the new method, preliminary experiments were repeated in which the presence of histaminase in the various tissues of the rat was determined. Of all the tissues examined, namely lung, liver, kidney, small intestine and blood, only lung and small intestine were capable of inactivating histamine.

It was then decided to study lung tissue alone. A series of experiments were carried out in which different amounts of lung tissue from normal rats was incubated as described above using the second method. A control was usually incubated at the same time. This consisted of a flask containing the histamine in a Buffer solution with added toluol but without any added tissue. Destruction of the enzyme by heating to 70°C for 5 minutes was also used as a control in some experiments. The results are given in Table XIX.

TABLE XIX.

Histaminase Activity of Lung Tissue from Intact Rats.

(Second Method).

Exp. No.	Amount of Tissue in grams	Histamine Destroyed in gamma	Remarks
147	0.05	200	
	0.1	300	
	0.6	825	
	1.0	875	
	Control	0	
151	0.05	200	
	0.1	300	
	0.5	700	
	1.0	875	
152	0.1	200	
	0.1	200	
	0.5	700	
	1.0	875	
	1.0	0	Heated to 70°C for 5 minutes
153	0.05	0	Heated to 70°C for 5 minutes
	0.1	200	
	0.5	400	
	Control	0	

It will be observed that in this series of experiments 0.5 gram of normal tissue will destroy between 700 and 800 gamma of histamine except in experiment No. 153, where 0.5 gram of tissue has inactivated only 400 y. If however one adds the five control specimens from the experiments on adrenalectomized animals (Table XX) this becomes less significant, since in all of these 800 y of histamine is destroyed by 0.5 gram of normal tissue. It will further be observed that the histamine is destroyed proportionately to the amount of lung tissue present. The results of different experiments vary slightly, but this is thought to be due to variations in the conditions of each experiment, such as temperature.

(b) The Effect of Adrenalectomy on the Histaminase Content of the Rat Lung:

In order to determine the effect of adrenalectomy upon the histaminase content of lung tissue, thirty rats were divided into two groups. The first group which consisted of ten animals was kept as controls. The second group of twenty animals were adrenalectomized at intervals as previously described and maintained on the usual Purina diet with 0.9% saline to drink. Eight to ten days after adrenalectomy groups of four adrenalectomized animals were killed and the lungs were removed. The lung tissue of these animals was then ground up and mixed. Varying amounts were used for histaminase determinations. At the same time, the histaminase content of the lung tissue from two animals of the group of controls was estimated. After three experiments had been done, it was decided to modify the treatment of the adrenalectomized animals as follows: The animals were maintained on Purina and 0.9% NaCl for six days. The saline was then replaced by water for three additional days, after which time the animals were killed and the tissues removed in the usual way. The results are shown in Table XX.

TABLE XX

Effect of Adrenalectomy on the Histaminase Content of Rat Lung Tissue.

(Second Method).

Exp. No.	Amount of Tissue in grams		Histamine Destroyed in gamma	Remarks
	Normal	0.5	800	
157	Adrenalect.	0.5	600	Animals adrenalectomized seven days. Maintained on Purina and 0.9% NaCl for 8 days.
	"	0.5	600	
	"	0.5	600	
	Normal	0.4	800	
158	Adrenalect.	0.2	300	"
	"	0.4	650	
	"	0.6	750	
	Normal	0.5	800	
159	Adrenalect.	0.2	300	"
	"	0.5	700	
	"	0.6	700	
	Normal	0.5	800	
	"	0.5	750	Animals adrenalectomized. Placed on Purina + 0.9% NaCl for 6 days. Then on water and Purina for 3 additional days.
164	Adrenalect.	0.5	400	
	"	0.5	450	
	"	0.5	300	
	Normal	0.5	800	
	"	0.5	700	
167	Adrenalect.	0.5	400	"
	"	0.5	400	
	"	0.5	300	
	"			

It will be observed that whereas normal lung tissue is able to inactivate approximately 1600 y/gm, lung tissue from adrenalectomized animals maintained on 0.9% saline will inactivate 1400 y/gm, a difference of 20%. It will be recalled that the weight of the lung of the rat increases by about 8% after adrenalectomy. This would therefore tend to make the actual amount of weighed tissue less, assuming that the gain in weight were due to added water, and would therefore make the actual difference in the histaminase content equal to approximately 12%.

If however the results obtained after placing the animals on water for three days are examined (Table XX) it will be observed that whereas the control tissue will inactivate 1600 y of histamine per gram, the adrenalectomized tissue is capable of inactivating only 800 y/gm, a difference of 50%. Since the difference in the average weight of normal lung tissue, and that of lung tissue from adrenalectomized animals maintained on water for three days has not been studied, it is impossible to state at present how much of this loss of histamine may be due to actual increase in tissue water.

(c) Attempts to Demonstrate the Mechanism of the Destruction of Histamine by the Kidney:

The previous experiments have shown that the rat kidney contains no histaminase as determined by the method just described. It was thought that the tissue might be capable of inactivating histamine already present in it such as occurs following an intravenous injection of histamine. Three experiments were carried out (see Table XXI).

In experiment 52 three animals were given an intravenous injection of 24 y of histamine per gram body weight. The kidneys were removed at one, two and three hours respectively. From previous experiments it is known that the average histamine content of the kidney following such an injection is 188 y/gm at the first hour, 17 y/gm at the second, and 4 y/gm at the third. These tissues were ground up separately and placed in beakers containing 20 cc of Buffer solution and 500 y of histamine. Toluol was added and they were then incubated for sixteen hours. In experiment 85 the kidney tissue was removed four hours following the histamine injection and incubated in the same way.

It will be observed that in all of these there was no destruction of the added histamine, whereas destruction of the histamine already present in the tissue removed at one hour seemed to occur (Exp. 52).

In a third experiment (No. 71) the kidneys of two animals were removed thirty minutes after the intravenous injection had been given. These tissues were incubated for 16 hours in Buffer solution alone. No histamine was added. It will be seen that whereas the assay of such tissue immediately upon removal yields an average histamine content of 450 y/gm (see Table IX) (p. 150) after incubation, the histamine content was only 176 y/gm in one and 388 y/gm in a second.

TABLE XXI.

Attempts to Demonstrate the Mechanism of the Destruction of Histamine by the Kidney.

(Animals injected with 24 y histamine per gram body weight. Kidney tissue removed at intervals indicated.)

Exp. No.	Time of Removal after Injection	Histamine Added in gamma	Histamine Found after Incubation in gamma	Average Controls in y/gm
52	1st hour	500	500	188
	2nd hour	500	500	17
	3rd hour	500	500	4
85	4th hour	500	500	0.9
	4th hour	500	500	0.9
71	30 minutes	0	176	450
	30 minutes	0	388	450

Discussion of the Foregoing Results.

Since the experiments thus far described have to do in the main with the inactivation of histamine by the animal organism and secondarily with the relationship of histamine of the adrenal gland to this process, they form a suitable division for discussion at this time.

From the experiments of previous workers, it has been shown that the organism as a whole may rapidly inactivate histamine as evidenced by the fact that a continuous intravenous infusion of the amine may be given to the experimental animal. (Jacobs and Mason 1936) or to human beings (Weiss, Ellis and Robb 1929) without producing any of its usual effects, providing that the rate of injection is slow.

This has been explained in part by the demonstration of the fact that the white blood cells, in particular the eosinophiles, are capable of taking up histamine (Code 1937b). It was also shown that if histamine were added to dogs' blood, it was taken up by the cellular elements, and that although the blood might have an excess of histamine, this extra histamine was physiologically inactive by virtue of the fact that it was held in combination with the cellular elements. That this inactivation was not due to a histaminase like action was shown by the fact that if the total histamine content of the blood were estimated, the added histamine could be recovered. (Anrep, Barsoum and Talaat 1936). While such a mechanism will explain why blood may contain fairly large amounts of physiologically inactive histamine, it does not account for the rapid disappearance of histamine from the blood as a whole.

Several possibilities suggest themselves. These are (1) the blood may inactivate histamine by some other mechanism than histaminase, or (2) the

tissues rapidly absorb the histamine from the circulating blood. These will now be discussed.

It has been shown by several workers that the blood of certain species is capable of inactivating histamine in vitro (Marcou 1938; Yen and Chang 1933).

In all the experiments done on the inactivation of histamine, it has been observed that histamine is destroyed or inactivated much more rapidly in vivo than in vitro. For example Marcou (1938) found that dogs blood was capable of inactivating small amounts of histamine when it was incubated with the amine for thirty minutes. In the first series of experiments described in this work, a very rapid disappearance of histamine from the blood after injection was observed, only 7.5% of the total amount injected being present after three minutes (Fig. 9) (p147a). Since the blood of the rat does not possess a histaminase like activity in vitro, it is assumed that this rapid disappearance must be due to some other factor.

Other mechanisms of histamine inactivation in the blood may however account in part for this rapid disappearance. For example it has been shown (Garan 1938a) that histamine may be inactivated by the action of carbon dioxide in vitro. Holtz and Heise (1937c) have observed that such substances as vitamin C, or compounds containing sulphur and hydrogen such as glutathione and cystein may also inactivate histamine in vitro under aerobic conditions, but on the other hand they point out that these substances will produce histamine from histidine if no oxygen is present. Holtz (1937) has also shown that histamine may be destroyed in vitro by simple oxygenation in the presence of a catalyst such as palladium. It is also interesting to note that Eichler and Speda (1938) were able to decrease the histamine content of the blood of cats by means of hyperventilation.

The second possible explanation to account for the rapid disappearance of intravenously injected histamine is that the tissues may absorb large amounts from the circulating blood. That this is so in the rat at least is borne out by the results of the experiments on the distribution of intravenously injected histamine described in the previous section. It was observed that following an intravenous injection of histamine in the rat, the distribution of the histamine detectable fifteen minutes after the injection was markedly in favour of the tissues. The amount of histamine recovered at fifteen minutes in all tissues was only 32% of the total amount injected of which only 2.9% was present in the blood.

Since it has been observed that most of the histamine is taken up by the tissues and that the kidney takes up by far the largest amount of histamine in comparison to the other tissues (approximately 50% of the total histamine recovered) and that this histamine disappears rapidly from the kidney after the first hour, several possible mechanisms for the destruction of the amine must be considered. These are (1) histamine may be excreted in the urine, (2) the main mechanism of histamine destruction is the histaminase action of those tissues which contain it and (3) there is some mechanism of histamine destruction other than histaminase. These will now be discussed.

The possibility that histamine may be excreted in the urine is not tenable in these experiments since in no case has any excretion of urine occurred before six to eight hours after such an injection of histamine. This has also been the observation of Howlett and Browne (1937), even when large amounts of saline were given intravenously.

Histamine has however been found in small amounts in the urine excreted by kidney in a heart-lung kidney preparation of the dog (MacGregor and Peat 1933). It has also been found in the urine of pregnant women by Ungar and Dubois (1937).

If one assumes that the main mechanism of histamine destruction in the rat is the histaminase action of those tissues which contain it, namely lung and small intestine, one might regard the kidney as a reservoir, taking up large amounts of histamine thus temporarily removing it from the circulation and then slowly liberating it to the histaminase containing organs for destruction. If this were the case, one might then expect the histamine content of the blood to decrease at a much slower rate or remain at a fairly constant level until such time as all of the histamine were destroyed. Examination of the blood histamine however does not confirm this. On this basis it is also difficult to understand why the liver which contains no histaminase, should lose its histamine content in the period fifteen to thirty minutes, whereas that of the kidney remains constant.

Assuming the presence of some mechanism other than histaminase in the rat kidney the fall of concentration in the other tissues during the fifteen to thirty minute period and the absence of any decrease in the kidney is due to the continued transfer of histamine from other tissues to the kidney at such a rate that the destruction of histamine by the kidney going on at the same time is not apparent. In the experiments on the mechanism of destruction by the kidney, it was noted that whereas this tissue was unable to inactivate histamine added in vitro, there was some destruction of the histamine already present in the organ (see Table XXI) (p. 179). A possible explanation may be found in the work of Holtz and Heise (1937c) already referred to. They showed that histamine could be inactivated by vitamin C and substances such as cystein and glutathione.

In other species however the kidney is known to contain large amounts of histaminase (McHenry and Gavin 1932b) and it has been shown in the dog that whereas histamine is only slightly inactivated when perfused through a heart-lung preparation it is rapidly inactivated when the kidney is

added to the circuit. (Best and McHenry 1930; MacGregor and Peat 1933; Essex, Steggerda and Mann 1935).

The liver also takes up a large amount of histamine in comparison to the rest of the tissues other than the kidney. (see Table X) (p. 152). Since the organ does not contain any histaminase, it would seem that some mechanism other than histaminase must be responsible for the destruction of the larger part of histamine introduced into the blood stream of the rat. This view was expressed by Best and McHenry (1931) and recently by Addarii (1937). This latter worker was also unsuccessful in trying to alter the histaminase content of guinea pig lung tissue by sensitizing the animals to horse serum and by pretreatment with histamine.

The amounts of histamine used in these experiments are larger than those which could in all probability be liberated under physiological or pathological conditions.

In the section on the relationship of the adrenal gland to histamine (p.115) it was pointed out that rats which have been adrenalectomized are much less resistant to histamine than is the intact animal (Wyman 1928; Marmorston-Gottesman and Perla 1931).

It is believed that the loss of resistance to histamine following adrenalectomy is due mainly to the absence of the cortex. Ingle (1937) has shown however that the medulla may also play a minor role.

The experiments on adrenalectomized animals confirm the finding that removal of the adrenal glands markedly decreases the resistance of the rat to histamine (see Fig. 10) (p.149), and that this decrease does not occur until at least seven days after removal of the glands. They indicate further that this decrease in resistance is due in part to a marked loss of ability of the organism to inactivate histamine. It is significant in this respect that the tissue mostly affected is the kidney since in the intact animal this

organ seems to be best able to inactivate histamine. It will be noted further that the distribution of intravenously injected histamine is not altered following adrenalectomy. Since the histamine content of the tissues are almost within normal limits at the end of three hours following the injection with the exception of the kidney and blood, it would seem that the effect of adrenalectomy is to retard the rate of inactivation.

In the experiments on histaminase it was shown that whereas adrenalectomy diminishes the histaminase content of the lung (see Table XX) (p. 176) the actual decrease is only about 12% in animals maintained on 0.9% saline. Since the adrenalectomized animals used in the experiments on the rate of disappearance of histamine were also maintained of 0.9% saline, the decrease in the ability to inactivate histamine could only have been due in small part to the diminished histaminase content of the tissues. This is further substantiated by the fact that the rate of disappearance from the lung in the adrenalectomized animal which contains histaminase is not as greatly affected as the rate of disappearance from the kidney which is devoid of histaminase (see Fig.10) (p.149).

Strict comparison between the results on the effect of adrenalectomy on the histaminase content of the lung and its effect on the rate of disappearance of intravenously injected histamine in normal and adrenalectomized rats is of course impossible.

The experiments on the effect of adreno-cortical hormone on the ability of the adrenalectomized rat to inactivate histamine demonstrate two things. Firstly, the so-called maintenance dose of cortin (0.5 cc daily) is quite inadequate to restore the ability of the adrenalectomized animal to destroy histamine even when the animals are maintained in good health on 0.9% NaCl. Secondly the decrease in the ability to destroy histamine occurring

in the adrenalectomized rat may be completely restored by the administration of sufficient amounts of adreno-cortical hormone. Whereas the hormone of the adrenal medulla may play some role in the immediate resistance of an animal to histamine, such as enabling it to maintain the blood pressure, it does not seem probable in the light of these results that it can play any part in restoring the ability of the adrenalectomized rat to inactivate or destroy histamine.

In the experiments on the effect of adrenalectomy on the histamine content of tissues, it is interesting to note that a marked increase occurs in the stomach and intestine. (Table VII) (p. 142). This increase is much greater in comparison to the decrease observed in the histaminase content of the lung, which is added evidence that some other mechanism besides histaminase which is capable of inactivating histamine exists in the organism.

It is also interesting to note that the increase in the histamine content of the stomach which occurs after adrenalectomy is as great as the amount of histamine absorbed by the stomach following the intravenous injection of a large dose of histamine (see Table IX) (p. 150).

The decreased resistance to histamine however is not regarded as evidence that the specific function of the adrenal is to render possible the destruction of histamine. The decreased ability to destroy histamine which develops only seven to ten days after adrenalectomy is however regarded as a part of the generally decreased resistance of the organism under these conditions which is evident even when the animals are maintained in apparently good health on sodium chloride (Selye 1937b).

Attempts to Demonstrate a Liberation of Histamine by Various Procedures, Utilizing the Histamine Content of the Kidney and Blood as an Index.

The observations of Selye (1937) have shown that if a rat is exposed to a damaging agent whether it be in the form of exposure to cold, excessive muscular exercise or adrenaline, a syndrome is produced, the symptoms of which greatly resemble those produced by the administration of large doses of histamine. Similarly the symptoms of traumatic shock have often been attributed to the liberation of a histamine-like substance (Dale and Laidlaw 1919).

Experiments described in the previous section have shown that histamine is rapidly removed from the blood (Rose and Browne 1938) and that the kidney taken up by far the greatest amount of the histamine introduced into the blood stream as compared to other tissues, it was thought that the histamine content of the kidney might serve as a much more sensitive index for the liberation of histamine in the organism, rather than that of the blood.

For this reason experiments were carried out on rats in the following manner. Groups of male and female rats were subjected to various damaging agents and the kidneys were removed at definite time intervals, namely 5, 10, 15, 30 minutes and 1 hour, and their histamine content estimated by the method previously described.

In the first series the animals were anaesthetized, and the stomach was exposed by means of a small abdominal incision. Using artery forceps, the stomach was then traumatized by clamping in three different areas for 30 seconds each. In order that the same degree of trauma might be inflicted in all rats, the same forceps were always used, and were always clicked to the first notch. Animals in which the kidneys were removed within fifteen minutes were kept anaesthetized. The other animals, namely those in which the

kidneys were to be removed at 30 minutes, and one hour, were allowed to come out of the anaesthetic, after closing of the wound. These were again anaesthetized before the kidneys were removed. The results are shown in Table XXII.

The effect of a subcutaneous injection of 0.5 cc adrenaline was studied on four groups and in one other that of an intraperitoneal injection of 0.2 cc of 4% formaldehyde. These animals were anaesthetized before removal of the kidneys (see Table XXIII).

TABLE XXII.

Histamine Content of Kidneys Removed at Various Times following Trauma to the Stomach.

(Values in gamma per gram).

Exp. No.	Time of Removal 5'	10'	15'	30'	1 hr
56	2.0	0.8	2.4	0.9	0.5
58	1.94	0.41	0.96	0.79	0.79
63	0.79	1.15	0.86	0.68	
63	0.72	0.85	0.78	0.40	
84	0.24	0.52	0.20	0.87	
87	0.55	0.384	0.58	1.79	

TABLE XXIII

Histamine Content of Kidneys Removed at Times Indicated following the Subcutaneous Injection of Adrenaline and the Intraperitoneal Injection of Formaldehyde.

(Values in gamma per gram).

Exp. No.	Time of Removal: 5'	10'	15'	30'	1 hr	Remarks
59	0.85	0.8	1.39	0.85	0.62	
66	0.43	0.66	0.43	0.67	0.67	Injection of 0.5 cc adrenaline
72	0.43	0.43	0.23	0.18	0.19	
73		0.57	0.33	0.35	0.02	Injection of 0.2cc of a 4% solution of formaldehyde

On examination of the results, it will be observed that an occasional increase in the histamine content of the kidney occurs. These are however very few in comparison to the number of insignificant increases. The results are variable, and if one takes the average normal histamine content of the kidney as being 0.54 y per gram with a variation of 0.02 - 0.9 y per gram (in one case 1.6 y) in only seven out of 40 estimations is the increase above 0.9 y per gram, although the general average is greater than that of normal kidney.

In another small group of animals the left kidney was first removed, and then the stomach was traumatized. After thirty minutes the right kidney was removed. The results are shown in Table XXIV.

TABLE XXIV

	KIDNEYS	
Exp. No.	Left (Control)	Right
90	0.6	0.59
and	0.9	0.97
92	0.65	0.67
	0.65	1.24
	0.9	0.42
	0.15	1.6
	1.6	2.0
	0.32	0.6

Legend: Left kidney first removed as control. Stomach traumatized and after thirty minutes, right kidney removed. Figures are given in histamine base per gram of tissue.

It will again be seen that the results are not consistent, a moderate rise occurring in 50% of the kidneys examined.

Finally, in two groups of animals, the histamine content of the blood was estimated following trauma to the stomach in one group of male rats, and after the intraperitoneal injection of 0.2 cc of 4% formaldehyde in a group of female rats. The results are shown in Table XXV.

TABLE XXV.

Effect of Trauma to Stomach and of an Intraperitoneal Injection of Histamine on the Blood Histamine Content of the Rat.

(gamma per cc).

Time of Removal	Exp. No. 63 Blood Histamine after Trauma to Stomach. (Male Rats)	Exp. No. 73 Blood Histamine after Formaldehyde. (Female Rats)
15 '	0.04	0.12
30'	0.03	0.10
1 hr	0.07	0.10
2 hrs	0.016	0.18
Control	0.02	0.07

It will be noticed that trauma to the stomach does not cause any appreciable increase in the histamine content of the blood within two hours after it is inflicted. There is a definite increase however in the blood histamine of the rats into which formaldehyde was injected.

In order to determine the relative amount of histamine which would have to be released into the blood stream so as to produce a significant rise in the histamine content of the kidney the following experiments were performed.

Histamine was injected intravenously into anaesthetized rats, starting with a small dose.in the first animal and gradually increasing the dose given to each subsequent animal in each case. The kidneys were removed thirty minutes after the injection of histamine had been given, and their histamine content was determined. The results are given in Table XXVI.

TABLE XXVI.

Experiments to Determine the Smallest Amount of Intravenously Injected Histamine Required to Produce a Significant Rise in the Kidney and Blood Thirty Minutes after the Injection.

p. No.	Amount of Histamine injected in mgm/gm Body Weight	Weight of Animals in grams	Total Histamine injected in mg	Kidney Histamine in y/gm	Blood Histamine in y/cc
34	0.0001	200	0.02	0.96	
	0.0002	200	0.04	2.9	
	0.0004	200	0.08	0.6	
	0.0006	195	0.12	0.9	
	0.0008	200	0.16	1.3	
	0.001	215	0.215	1.25	
	0.003	200	0.6	1.35	
	0.005	180	0.9	2.3	
	0.01	170	1.9	6.2	
	0.02	200	3.4	84.5	
		190	Control	0.3	
35	0.0005	165	0.082	0.2	
	0.001	155	0.155	0.4	
	0.002	155	0.31	2.0	
	0.004	150	0.6	5.0	
	0.006	155	0.93	16.0	
	0.01	155	1.55	12.3	
	0.013	155	2.0	19.5	
	0.016	180	2.8	58.2	
			Control	0.4	
			Control	0.35	
			Control	0.15	
			Control	0.35	
110	0.0005	150	0.082	3.1	0.13
	0.001	165	0.155	4.2	0.11
	0.0015	165	0.232	3.1	0.06
	0.002	165	0.310	4.1	0.10
	0.003	165	0.465	5.0	0.15

It will be observed that the first significant increase in the histamine content of the kidney occurs with an approximate dose of 0.0015 mg/gm body weight. In an animal weighing 150 grams this would be 0.225 mgm of histamine.

Discussion:

Although a slight increase in the histamine content of kidney of the rat occurs following exposure of this animal to some harmful stimulus, the results are not consistent.

It is improbable that the liberation of such small quantities of histamine as 0.25 mg into the circulating blood, or the slight accumulation of histamine in an organ such as the kidney could be of very great significance in the production of the immediate symptoms which may occur following the administration of a damaging stimulus to the rat.

It is possible however that damage to an organism may produce a slow liberation of histamine which may be of significance if other factors are present such as alteration in the pH of the blood etc.

CLINICAL INVESTIGATIONS.

(A) Blood Histamine:

With the development of a method for the estimation of histamine in the blood, a new field of investigation was opened up and a number of interesting observations were made. (Code and MacDonald 1937) (Riesser 1937).

Although the greater part of the experimental investigations here presented has been carried out on animals, opportunity was afforded from time to time to examine the blood of patients on the wards of the Royal Victoria Hospital. Many of these were taken at random, but several groups of patients suffering from the same disease have also been included. A consideration of these will now be presented.

(a) Blood histamine in normal invididuals:

The blood histamine of eleven normal adult individuals, five male and six female, was estimated. The results are given in Table XXVII. The average figure from this small group is approximately 0.04 y/cc. This is in agreement with the results of Haworth and MacDonald (1937) who obtained an average of 0.04 y/cc with a range of from 0.018 to 0.078y/cc in a series of 103 determinations on students.

TABLE XXVII

Blood Histamine Content of Normal Individuals.
(in gamma per cc)

No.	Male	No.	Female
1	0.04	7	0.05
2	0.05	8	0.04
3	0.04	9	0.04
4	0.06	10	0.05
5	0.035	11	0.03

(b) Histamine content of the blood of pregnant women:

In 1937 Ungar and Dubois reported the presence of a histamine-like substance in human pregnancy urine. This substance could not be found in the urine of non-pregnant women or in that of normal men (Ungar and Pocoulé 1937). It was decided therefore to examine the blood histamine content in a group of pregnant women with normal pregnancies and in those manifesting the signs and symptoms of toxemia.

The results are shown in Table XXVIII.

Examination of these results does not reveal any significant difference in the histamine content of the blood of these patients as compared with that of normal individuals, although there is a definite increase in a few cases.

TABLE XXVIII

Histamine Content of Blood of Pregnant Women.

(in gamma per cc)

Pregnant Females		
No.	Duration of Pregnancy	Histamine
34	5 months	0.09
42	5 "	0.06
39	6 "	0.05
35	7 "	0.05
38	7 "	0.08
40	7 "	0.10
41	7 "	0.07
54	8 "	0.04

Pregnant Females		Toxic
No.	Duration of Pregnancy	Histamine
32	Term	0.04
33	"	0.04
36	"	0.11
55	"	0.06

(c) Blood histamine of patients with various diseases:

The blood histamine was determined in a group of fifteen patients taken from the wards of the hospital at different times. The results are shown in Table XXIX. A definite increase in the histamine content of the blood is observed in four of these patients. No. 23, a case of cardiac insufficiency, No. 44, a patient during an attack of vasomotor rhinitis, No. 48, a girl in an acute attack of urticaria and No. 62 a patient with lymphatic leukaemia, in whom the blood was taken within five minutes after X-ray treatment. It is interesting to note that a decrease in the blood histamine seems to occur in patients during various types of shock.

TABLE XXIX

Blood Histamine in Patients with Various Conditions.

No.	Diagnosis	Remarks	Histamine
23	Cardiac Decompensation		0.11
26	Shock	Surgical	0.02
59	Shock	Severe Traumatic	0.008
47	Shock	Acute Coronary After Recovery	0.02 0.09
60	Shock	Pneumonic Crisis Convalescent	0.01 0.05
53	Convalescent Pneumonia		0.09
37	Augioneurotic Oedema	After Attack	0.02
44	Vasomotor Rhinitis	Attack during Menses	0.16
61	Asthma	Quiescent	0.01
52	Asthma	Quiescent Severe Attack	0.04 0.02
30	Menopausal Syndrome	Quiescent	0.04
28	" "	"	0.05
43	Scleroderma	Eosinophilia	0.04
48	Urticaria	In Acute Attack	0.20
62	Lymphatic Leukaemia	5' after X-Ray treatment	0.28

(d) The release of histamine into the blood following various forms of stimulation to the skin:

Lewis (1927) first postulated that the reaction which occurs following stimulation of human skin, i. e. the triple response, was due to the liberation of an 'H' substance (histamine). This has been confirmed indirectly by Kalk (1929) and others. Using the method of Barsoum and Gaddum (1935a) Cerqua (1936) first demonstrated that an increase of the blood histamine occurred in patients during an attack of urticaria.

Since many patients afflicted with urticarialattacks frequently have so-called sensitive skins or dermatographia, it was decided to study the blood histamine before and after mechanical stimulation to an area of skin, usually the back, and by other forms of stimulation such as cold and heat.

The experiments were carried out as follows:
A control specimen of blood was first taken. In three patients an area on the back of about the size of a hand was stimulated by scratching vigorously for about thirty seconds. The usual response of red line and flare was produced bu wheals were not formed. Blood specimens were taken at the times indicated, and it should be noted that these would then indicate the histamine content of the general circulation since the stimulated area was not on the arm from which the blood specimens were removed.

In others an arm was placed in cold or hot water and the blood specimens were taken from the same arm as indicated. The results are given in Table XXX.

TABLE XXX

Release of Histamine in the Blood of Patients following Various Forms of Stimulation.

Patient	Diagnosis	Type of Stimulation	Time	Blood Histamine in y/cc
28	Urticaria	Hand and Wrist in Ice-Water 3°C	Control after 20'	0.03 0.09
48	Urticaria	Hand and Wrist in Ice-Water 3°C	Control after 20'	0.03 0.14
51	Urticaria	Back Scratched Vigorously (Moderate Reaction)	Control after 5' after 20'	0.08 0.16 0.02
56	Vasomotor Rhinitis	Back Scratched (Mild Reaction	Control after 5' after 15'	0.10 0.10 0.14
58	Sensitivity to Heat (Urticaria)	Hand and Wrist in Water at 55° C	Control after 5' after 15' after 30'	0.10 0.14 0.10 0.10
49	Urticaria Factitia	Back Scratched (Marked Reaction)	Control after 5' after 20'	0.07 0.40 0.10

It will be observed that an increase in the blood histamine occurs usually within five minutes of the stimulation. This is quite marked in case No. 49 where there is an increase of five and a half times the normal.

Discussion:

Increases in the histamine content of the blood of patients have been observed under different conditions. For example Barsoum and Smirk (1936a) noted an increase in the histamine content of plasma during reactive hyperemia. They believed that the production of the hyperemia was in part due to the liberation of this histamine.

No investigations have as yet appeared on the histamine content of the blood of patients in shock due to trauma or other conditions. The weight of evidence from animal experimentation is in favour of the theory that little or no increase takes place. (Holt and MacDonald 1936; MacDonald and Woolfe 1938). These investigators used the method of Barsoum and Gaddum (1935a). Using a different method however Minard (1937b) was able to show an increase of 80% of the normal.

While these experiments are of an acute nature, no experiments on shock of longer duration have been done. The results obtained on three patients, all of whom presented the clinical picture of shock seem to indicate that the histamine content of the blood is,if anything, decreased. (Table XXVI, p. 195). One of these, however, was a case of acute coronary thrombosis (Pat. No. 47), and here one must make a distinction. It has been pointed out (Eppinger 1934) that cases of 'true' shock must be differentiated from those cases of shock due to cardiac failure. In the first type, the venous blood pressure is low, and the veins are collapsed. Arterial pressure is lowered, and the blood volume is diminished. Cases of heart failure on the other hand differ in that although the arterial blood pressure is also decreased, blood volume is usually increased, the veins are distended,

and venous blood pressure is high. It is believed by some (Rusnyak, Karady and Szabo 1936) that the production of shock other than that due to cardiac failure may in part be due to the liberation of histamine.

The results obtained here are not necessarily against this view, since it is known that liberated histamine is rapidly 'fixed' by the tissues at least in the rat. (Rose and Browne 1938a).

The relationship of histamine to asthma has been discussed in a previous section (p.). Studies on the histamine content of the blood of asthmatic patients have been carried out. Haworth and MacDonald (1937) found no significant increase in a series of seventy-eight patients. Riesser (1937) was unable to demonstrate any increase either in the resting state or during an attack. Cerqua (1935) and Parrot (1938) both report an increase in the histamine content of the blood of asthmatic patients during the height of an attack.

An increase in the histamine content of the blood of patients suffering from myeloid leukemia has been reported by Code and MacDonald (1937). Since it was shown by Code (1937d) that the source of histamine in the blood is the white blood cells, this is not surprising. Increases of up to 15.0 y/cc were observed (normal blood histamine 0.04 y/cc). This well illustrates the fact that a marked increase may occur in the blood histamine without any physiological action taking place provided that the histamine is not free in the plasma. Code and MacDonald (1937) showed that there was no significant increase in the histamine content of the blood of patients with lymphatic leukemia. It is believed therefore that the increase in the blood histamine of patient No. 62 (Table XXVI, p. 195) is due to the effects of X-ray rather than the lymphatic leukemia.

Although no definite change is seen to occur in the blood histamine of pregnant women, up to the eighth month of pregnancy, it is significant that

the histamine content of the blood of patients who are toxic are still at normal levels at term. It has recently been shown by Marcou et al (1938) that the histamine content of the blood of pregnant women rapidly diminishes at term. This is supposedly due to the production of histaminase by the placenta, and the appearance of histaminase in the blood.

The theory that histamine may play a role in the mechanism of labour is in part supported by the work of Danforth (1939). He found an increase in the histaminase content of the placenta in patients with uterine inertia, indicating that the mechanism of the inactivation of histamine must be increased, resulting in a decrease in available histamine. Danforth (1929) however does not believe that the evidence warrants the conclusion that this lack of histamine is a factor in the production of uterine inertia.

The release of histamine into the blood of patients subject to urticarial attacks following stimulation of the skin supports the view of Lewis (1927) that stimulation or injury to the cells of the skin result in a release of histamine. This theory has recently been opposed by the work of Menkin (1938b). This latter worker believes that the increase in permeability of the capillaries, and the formation of oedema which occurs in inflammation is due to another substance which he has called 'leucotaxine.

(see p. 98).

(B) Histamine Sensitivity:

In 1934, Karady in an endeavour to determine the sensitivity to histamine in patients, devised the following test. He injected a small dose of histamine intravenously, and followed the changes produced in the blood pressure. On examination of the resulting blood pressure curves, four main types could be distinguished. This test was then performed on a large number of patients about to undergo major surgical operations, and it was noted that the patients which tended to develop postoperative shock fell into the same group as determined by the test, that is, Type II. Rusnyak, Karady and Szabo (1936) found further that if such patients (Type II) were pretreated with histamine subcutaneously for eight to ten days, the response to the intravenous injection of histamine was modified, and it was also noted that such pretreatment prevented the onset of surgical shock following operation.

In order to explain the protective action afforded by histamine pretreatment, Rusnyak, Karady and Szabo (1936) and Karady (1938) performed experiments on animals in which it was shown that pretreatment of an animal with histamine will prevent the decrease in blood volume which usually occurs during histamine shock, or shock due to surgical trauma.

It seemed of interest therefore to repeat the first part of the work, in order to evaluate these results.

In collaboration with Dr. S. Baxter and Dr. S. Karady, a series of 160 patients taken from the medical and surgical services of the Royal Victoria Hospital were examined.

Method:

A solution of histamine containing 0.003 mg/cc (3 y/cc) is made and put up in sterile glass ampoules. In order to carry out the test two cc (6 y)

of this solution are injected into the median basilic vein of one arm. The systolic blood pressure is followed every 5 to 10 seconds in the opposite arm. Two people are necessary to carry out the test, one of whom takes the blood pressure readings, while the other injects the histamine solution. Certain precautions should be taken. The blood pressure of the patient should be stable before making the injection and the histamine solution should be injected rapidly.

Such an amount of histamine frequently causes flushing of the face and neck, a metallic taste is felt and an increase in the pulse rate is observed.

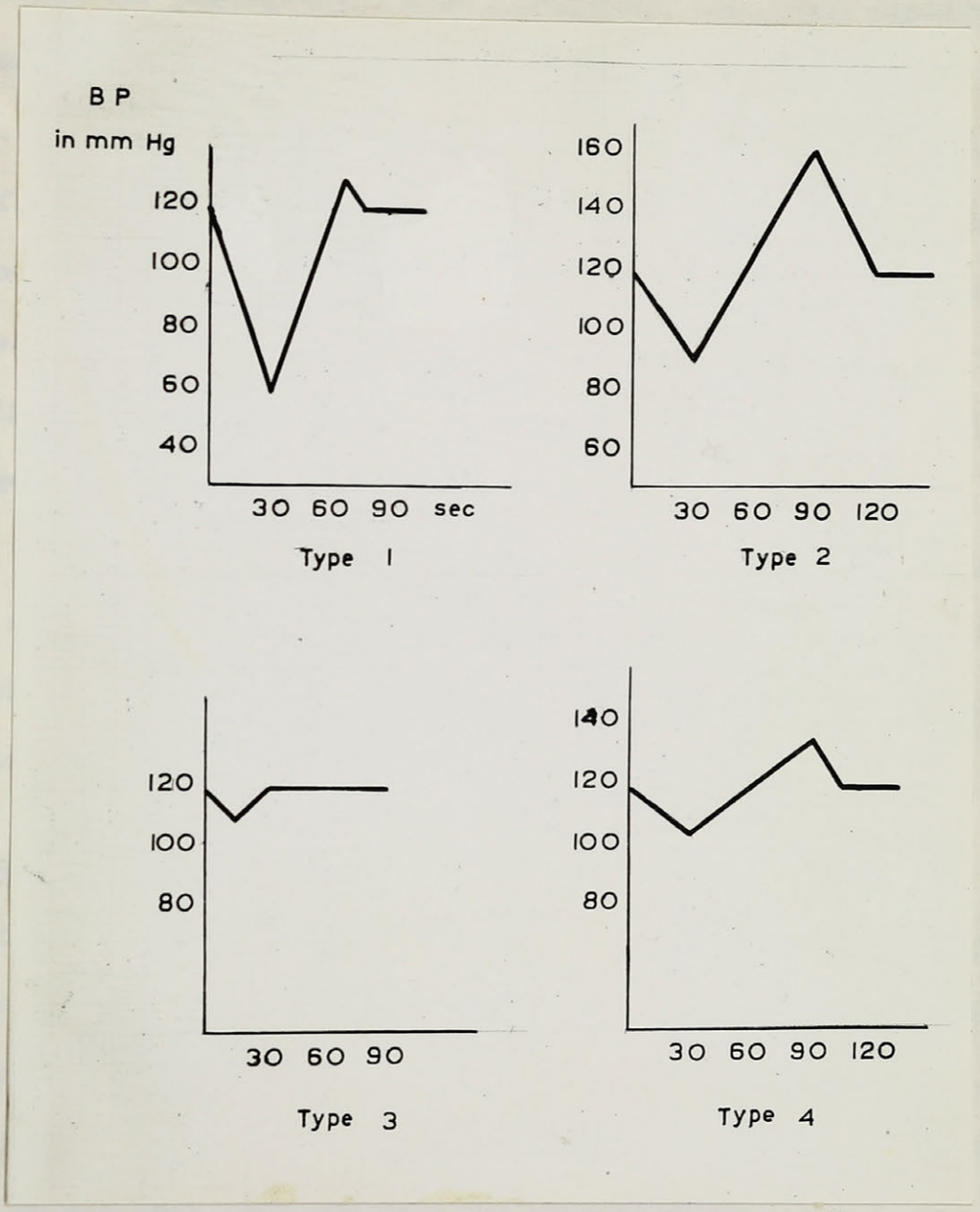
The changes which occur in the blood pressure are shown in the specimen curves (Fig. 12). Four types of blood pressure responses were found. Type one is characterized by a drop of 20 - 40 - 60 mm Hg. within thirty seconds, which is followed by a return to normal or a slightly higher point within one minute and a half. This type of response is found in the majority of individuals. Type two is characterized by an initial drop of at least 20 mm of Hg. followed by a return to normal and a secondary rise of at least 20 mm Hg, over the original level, with a final return to normal.

To type three belong those individuals whose blood pressure response is slight in that a drop of 5 to 10 mm Hg followed by a return to the original level occurs.

Type four is characterized by a drop of less than 20 mm Hg followed by a return to and a secondary rise of less than 20 mm Hg over the original level.

Of the patients examined 65% fell into Type I, 7% were of the second type, 9% were of the third type, and 6% fell into Type IV. The remaining 13% made up cases of hyperthyroidism and these will be discussed separately.

Figure 12



Showing the Response to an Intravenous Injection of Histamine (0.006 mg) in Patients.

Of fifty -nine surgical cases, only six belonged to Type II, and in none of these did surgical shock develop.

Two cases of urticaria in whom a Type II response was obtained were chosen for pretreatment with histamine. They were treated as follows: 0.005 mg of histamine subcutaneously the first day and 0.01 mg on the second. The dose was then increased by 0.01 mg of histamine until a daily dose of 1.0 mg was attained. This was continued for three days, and then the blood pressure test was given again. In both cases the response to the intravenous injection of histamine was altered (see Fig. 13). Instead of the original Type II response, the blood pressure now gave a modified Type I response.

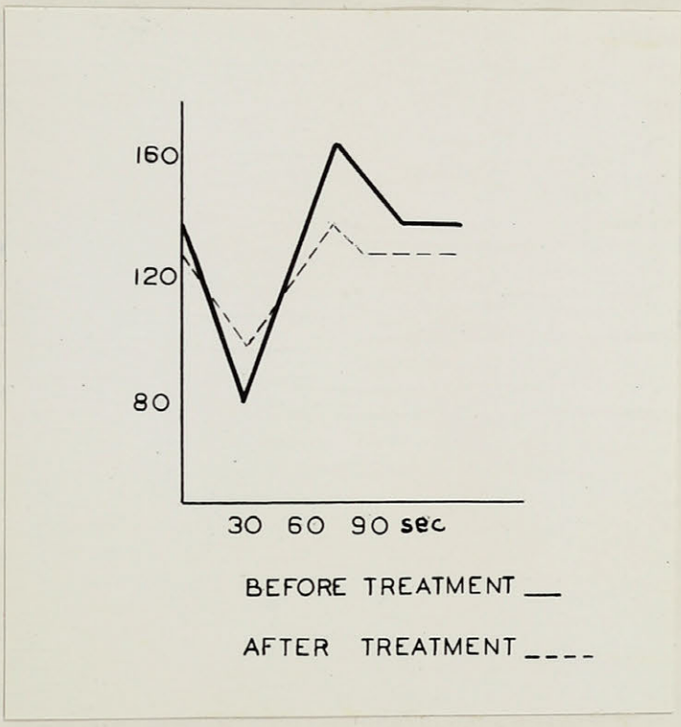
Cases of hyperthyroidism usually give a very mild response, in that a slight fall in the blood pressure (5 mm Hg) occurs. It is believed that this is due to the fact that the capillaries are already dilated, and as a result, the action of histamine is greatly diminished.

Discussion:

While it is probable that the response of the blood pressure to an intravenous injection of histamine may be an indication of the degree of sensitivity to histamine the results of such a test are not in agreement with those found by means of a subcutaneous injection of histamine. For example it has been reported that the skin sensitivity test to histamine is increased in cases of asthma (Ramirez and George 1924), however, blood pressure response to an intravenous injection of histamine does not fall into the Type II group, in such cases.

There are certain points which deserve attention however. According to Rusnyak, Karady and Szabo (1936) surgical shock is produced in part by the liberation of histamine, and this explains why these patients go into shock. The greatest argument against such a theory is that up to the present

Figure 13



Showing the Effect of Histamine Pretreatment on the Blood Pressure Response to an Intravenous Injection of Histamine.

it has not been possible to demonstrate a release of histamine into the blood of animals in which acute traumatic shock has been produced (MacDonald and Woolfe 1938). If however one considers the amount of histamine which will produce a drop of blood pressure in the human being this is not surprising. The normal histamine content of the blood in man is 0.04 y/cc. If one injects six gamma intravenously, a marked drop of blood pressure may occur as has been shown. For example: In one patient with hypertension and arterio-sclerosis, a drop of over 100 mm Hg was produced (205 - 100 mmHg). If one assumes the average blood volume to be about 6000 cc the addition of 5 y to this when diluted adds 0.0007 y/cc., an amount impossible to measure. Further difficulty is added by the rapidity with which histamine disappears from the blood.

Experiments on the Rapid Production of an Increased Resistance to Histamine in the Cat.

In the experimental work just described it was shown that the blood pressure response to an intravenous injection of histamine can be altered in the human being by pretreatment with histamine (see Fig. 13, p. 210a In order to explain this mechanism, Karady (1936) treated cats with subcutaneous doses of histamine for a period of eight to ten days. He observed that whereas untreated animals reacted in the usual manner to an intravenous dose of histamine (10 y) i. e. a marked drop in the blood pressure, in animals treated for three to four days the drop in blood pressure was much less, and in animals treated for a period of eight days, a slight rise in the blood pressure occurred when a similar dose (10 y) was given intravenously

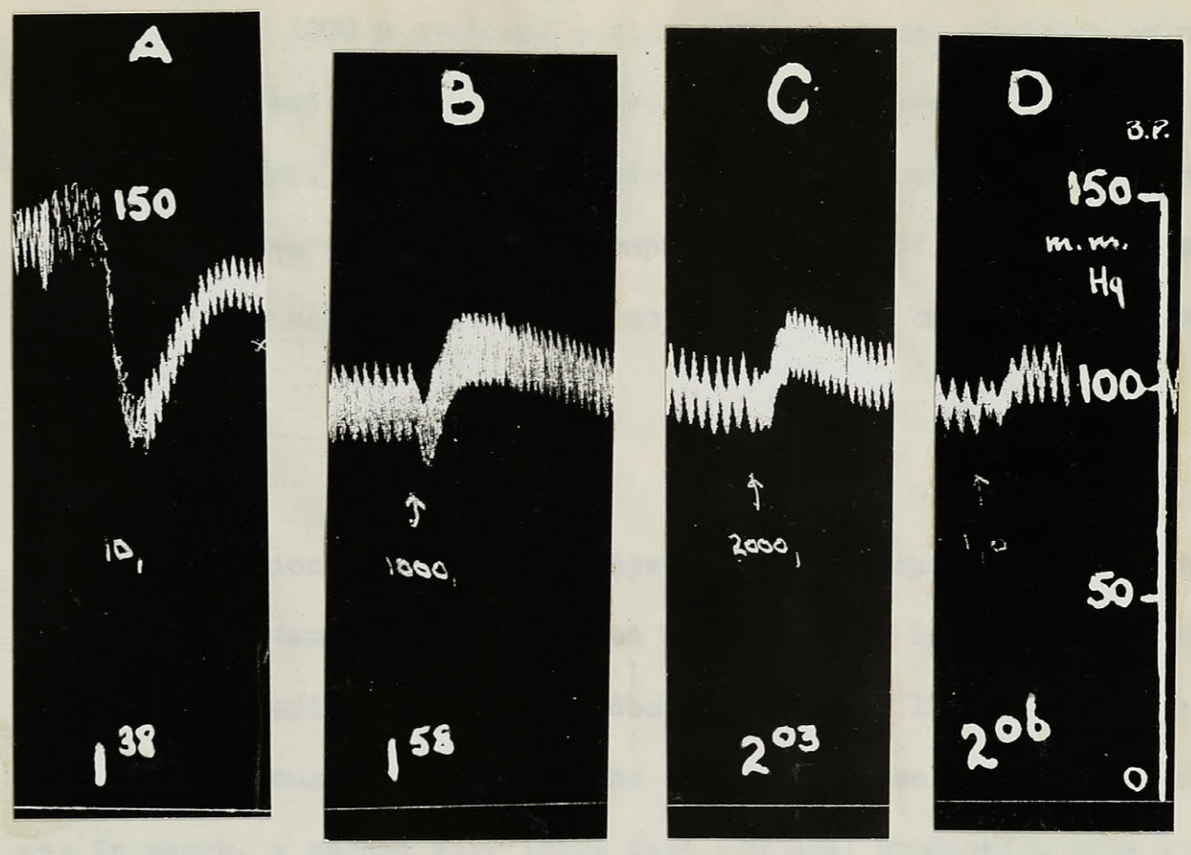
Karady (1936) further observed that if frequent intravenous doses were given to an anaesthetized cat, the effect on the blood pressure was gradually altered from a depressor to a pressor response.

In collaboration with Dr. Karady these last experiments were repeated in a group of six cats weighing between 1.8 and 2.5 kgs.

The animals were anaesthetized with dial (Ciba) and prepared in the usual manner. Blood pressure was recorded from the carotid artery, and the injections were given into one femoral vein. (see methods p.).

Histamine injections were then given at intervals of two to three minutes. The doses were rapidly increased. A reversal of the usual response to histamine was obtained in all animals when a dose of 1000 to 2000 y was reached. In all except one of the six animals the blood pressure was maintained at or above 100 mm Hg. An example of one of these experiments (No. 5) is shown in Fig. 14. The protocol shows the various doses given and

Figure 14



PROTOCOL I

Exp. 15 Male Cat Dial Anaesthesia

Time	Histamine Injected in y		B. P.
1 30			150
1 34	1		
1 38	10	(A)	
1 40	50		
1 44	100		
1 48	200		
1 52	500		
1 54			
1 57	1000	(B)	100
1 59	1000		
2 03	2000	(C)	100
2 06	1	(D)	100

- - - - - - - - - - - - - - -

the specimen tracings are indicated by the letters. It will be observed that a dose of 1000 y produces a rise with the blood pressure maintained at 100 mm Hg (B) and that a slight rise is obtained even with 1 y of histamine (D)

In Exp. 3 (Fig. 15) however, while similar rises were obtained in the blood pressure following the rapid injection of successive doses of histamine, the blood pressure was not maintained, but fell to a level of 45 mm Hg.

Discussion:

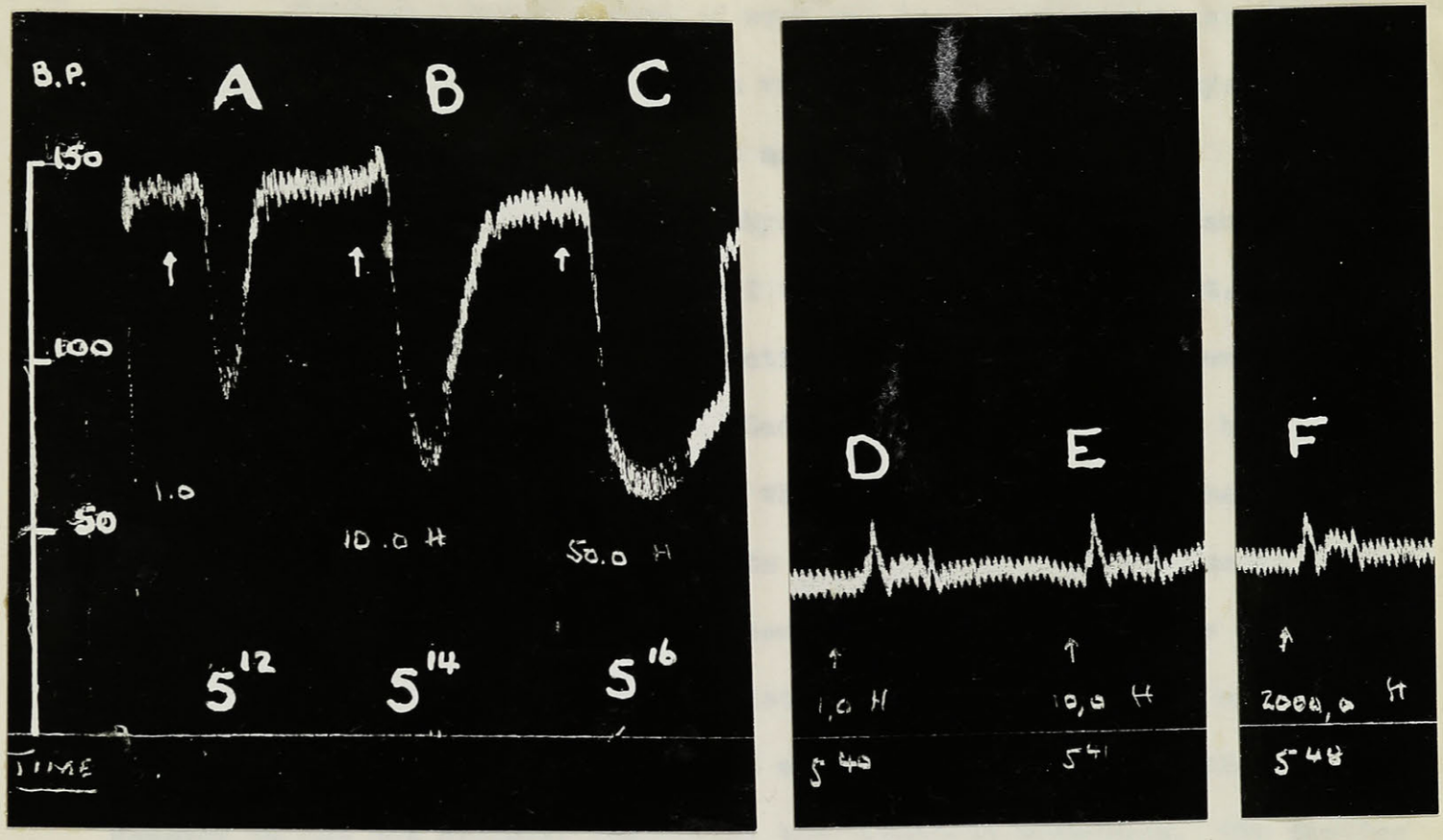
Previous workers have shown that the response to an intravenous injection of histamine may be altered by a previous intravenous injection (Meakins and Harington 1923; Feldberg and Schilf 1930). Using the cat, a large intravenous dose of the amine was first given (5 mgm). When the animal was in shock, a second very large dose (10 mgm) produced a rise in blood pressure. This may be explained by the fact that the first dose of histamine paralyses the capillaries, and therefore the action of histamine on the arterioles, that is vaso-constriction, becomes dominant, with a resulting rise in blood pressure. It should be noted that these animals were in shock.

In the experiments just described on the other hand, a tachyphylaxis or rapid production of resistance is produced, as evidenced by the fact that blood pressure is maintained and further that a pressor effect is obtained from doses as small as 1 y. (see Fig. 14).

It would seem therefore that the mechanism is more of desensitizing nature than a paralyzing one.

Similar observations have been made by other workers (Eichler and Killian 1931) using the rabbit.

Figure 15



PROTOCOL II

Experiment 3 Male Cat Dial Anaesthesia

Time	Histamine injected in y		B. P.
5^{00}			150
5^{12}	1	(A)	
5^{14}	10	(B)	
5^{16}	50	(C)	
5^{18}	50		
5^{20}	100		
5^{24}	200		
5^{26}	500		
5^{29}	1000		120
5^{34}	1000		
5^{36}	1000		
5^{38}	1000		
5^{40}	1	(D)	45
5^{42}	10	(E)	45
5^{43}	50		

cont'd

Time	Histamine injected in y		B. P.
5^{44}	100		
5^{45}	200		
5^{47}	500		
5^{48}	1000	(F)	45

- - - - - - - - - - - - - - - - - - - -

Final Discussion:

Although a large amount of work has been performed in an effort to determine the role of histamine in various conditions, the physiological significance of this substance is not as yet clear.

Perhaps the most probable physiological action of histamine is concerned with the local regulation of the blood supply to a part. It would then by means of its vaso-dilator reaction increase the blood flow. In the introduction to the monograph by Gaddum and Dale (1936) Dale has expressed the view that histamine and the other tissue-hormones may act collectively as the natural antagonists to the vaso-constrictor mechanism which is primarily controlled by the central nervous system. The work of Ungar (1938) seems to indicate that histamine may be released by means of a nervous mechanism similar to that by which acetyl-choline is liberated. However he has not properly identified the liberated substance. The finding that the histamine content of the blood may be increased by anoxemia (Barsoum and Gaddum (1935b) and decreased by hyperventilation (Eichler and Speda 1938) may possibly be correlated with some regulatory vascular mechanism.

There seems to be some mechanism in the organism by which the histamine content of the tissues and of the blood is kept at a constant level. For instance, histamine which is formed in the intestinal lumen must for the most part be inactivated as it passes through the wall of the intestine. Similarly increased amounts of the amine liberated into the blood are taken up by various tissues to be destroyed there. There are several ways in which a liberation of histamine can be dealt with, depending upon the site and cause of liberation. Histamine which is liberated into the tissue spaces by cell stimulation or injury exerts a local effect. This may be regarded in part as a protective mechanism, for if all the histamine

were suddenly to enter the blood stream, a marked general reaction would result.

That some of this histamine may enter the blood stream after local tissue damage has been shown in the experiments previously described (p.203

From the recent work of others one may postulate that under normal conditions, a certain amount of histamine is constantly being produced, most probably from histidine. Under pathological conditions such as anaphylactic shock however it has been shown that large amounts of histamine present in the tissues in loose combination may be liberated into the blood stream. There are several ways in which the organism is protected against the action of this sudden liberation.

First, there is the immediate removal of histamine from the circulating blood plasma into the white blood cells where it becomes inactive, but not destroyed. Secondly, there is the rapid withdrawal of the histamine from the blood into the tissues where it becomes fixed, and finally is destroyed.

It has been shown that the presence of the adrenal cortex is essential to the maintenance of an ability to destroy histamine. This is illustrated by the fact that a marked increase in the histamine content of certain organs occurs following the removal of the gland, and that further the ability to inactivate histamine is markedly affected. This is not thought to be the specific function of the adrenal gland, but is rather regarded as part of the general resistance maintained by the presence of the adrenal cortex.

The mechanisms of destruction have been discussed, and whereas it is felt that histaminase may be an important factor in maintaining the normal amounts of histamine in blood and tissues, it cannot account for the rapid destruction which takes place in the tissues when large amounts of

the amine are liberated into the blood stream.

The finding of histamine in the gastric juice by MacIntosh (1938) has led him to believe that histamine may be a normal stimulant to gastric secretion, although he is not of the opinion that it is the 'gastric hormone'.

The recent work on histaminase in the human placenta is interesting but as yet one cannot say whether or not histamine plays a physiological role in pregnancy or in the mechanism of child birth.

The use of histaminase preparations, and of active histamine desensitization have been successfully used as protective mechanisms against the action of histamine in animal experimentation. The possibility of such procedures being of therapeutic value clinically is supported by the successful treatment of patients who are sensitive to cold (Horton, Brown and Roth 1936).

SUMMARY.

The investigations reported in this thesis have led to the following finding: -

1. Studies on the histamine content of the tissues of the rat have been carried out. Of all the tissues studied, the stomach contains the greatest amount of histamine.

2. A sex difference in the histamine content of the blood of the rat was found. This histamine content of the whole blood of the normal female rat is higher than that of the male rat.

3. Adrenalectomy causes a marked increase in the histamine content of the stomach and small intestine.

4. An increase in the histamine content of the lung and liver occurs after ovariectomy.

5. Studies on the fate of intravenously injected histamine have shown that the distribution of histamine injected intravenously into the intact and adrenalectomized rat is markedly in favour of the tissues as compared with the blood within fifteen minutes.

6. Of the tissues studied the kidney takes up most histamine.

7. Although the concentration of histamine in the blood, lung, liver and lymph glands has begun to decrease within fifteen minutes after the injection, no apparent decrease is evident in the kidney up to the thirty minute period. After this time the rate of disappearance is greatest in the kidney.

8. Adrenalectomy has little effect on the initial tissue distribution of histamine injected intravenously in the rat, but markedly retards the rate of its disappearance.. This effect is most marked in the kidney.

9. Although the ability of the adrenalectomized rat to inactivate histamine at the normal rate is restored by the administration of large doses of cortin or desoxycorticosterone, the normal maintenance dose of cortin is quite inadequate.

10. Studies on the histaminase content of the tissues of the rat in collaboration with Dr. Karady show that only lung and small intestine contain histaminase. It has also been found by the author that the histaminase content of the lung tissue of the rat is decreased following adrenalectomy.

11. It has been shown by the author that histamine may be released into the blood of patients following stimulation of the skin, by scratching or by exposure to heat or cold.

12. The work of Karady on sensitivity to histamine by means of an intravenous injection of histamine in patients has been repeated.

13. The existence of a tachyphylaxis in the cat has been confirmed.

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