CHANGES IN HISTAMINE CONTENT OF GUINEA-PIG ORGANS UNDER VARIOUS EXPERIMENTAL CONDITIONS

BY

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INTRODUCTION

Histamine, a naturally occurring substance has been identified, located and traced through its metabolism. It is very widely distributed in the body and there is scarcely any organ which does not contain at least some histamine.

Abnormal release of this substance or altered reactivity to histamine has been found to occur in many pathological conditions. However, the great amount of information which has accumulated over the years, has contributed very little in understanding the role, that is the physiological function, if any, of this biological amine.

In 1960 KAHLSON suggested that histamine has an important role in tissue growth. He based this theory on experiments performed on rats and found that during embryonal development and in regenerative and reparative growth, the histamine-forming capacity of the tissues involved is greatly increased. He postulated that histamine probably plays an active role in tissue growth and regeneration. Some aspects of KAHLSON's work have been confirmed, but others are still controversial.

Earlier published studies concerning the amount of histamine in different body tissues under different experimental conditions have led to variable and conflicting results. It was therefore thought that studies along these lines using 'model' animals with high or low histamine tissue contents, might be a fruitful

approach to the problem. The guinea-pig was chosen as the test animal in these studies, since it is very sensitive to the effects of histamine and its reactions in this respect have been considered to be closest to those of man.

It is therefore the purpose of this work to induce changes in the tissue histamine content of guinea-pigs, in the hope that such a study might prove useful in understanding the physiological role of histamine in the body and its significance in influencing pharmacological responses to drugs.

I LITERATURE REVIEW

A. Histamine. Historical Introduction.

SCHMIDT-MULHEIM (1880) described a state of shock that occurred in an experimental dog after it had received an intravenous injection of a proteose-peptone mixture which was known as "Witte's peptone". Those studies were the first concerned with the phenomenon histamine release, although at the time unrecognised. PORTIER and RICHET (1902) first described the phenomenon of anaphylaxis. The presence of a depressor substance in tissue extracts, was also early noted by VINCENT and SHEEN (1903).

Histamine was first obtained by WINDAUS and VOGT (1907), who prepared synthetically β-imidazolyl-ethylamine (histamine) from imidazolyl-propionic acid (histidine). They were, however, unaware of the biological importance of their compound until three years later, when DALE and LAIDLAW (1910) published a detailed study of the pharmacological effects of histamine. BARGER and DALE (1910a) and KUTSCHER (1910) also showed that histamine is a constituent of ergot. BARGER and DALE (1910b) the same year isolated histamine from the intestinal mucosa, and MELLANBY and TWORT (1912) reported that organisms usually present in the intestinal tract of man and animals effect the decarboxylation of histidine, and that histamine is a constituent of the intestine and faeces.

DALE and LAIDLAW (1910 and 1911) suggested the possibility that histamine may play a causative role in the phenomenon of anaphylaxis. These authors had observed the similarity of the immediate symptoms of anaphylactic shock with those elicited by doses of histamine. It was in these early years that histamine gave evidence of being a causative agent in a number of pathological conditions.

LEWIS (1927) found that a large variety of mildly injurious stimuli on the human resulted in a series of responses which he termed "triple response", a reaction which he postulated was caused by the liberation of a substance similar to histamine (H-substance).

DALE (1929) reaffirmed his initial views, supported by LEWIS' observations, and formed the theory that a combination of antigen and antibody results in tissue injury which liberates free histamine from an inert bound form.

An important step in the study of the distribution of histamine in living tissues and biological fluids was the method described by BARSOUM and GADDUM (1935) and modified by CODE (1937).

WERLE (1936) first reported that histamine could be formed in animal tissues by the enzyme histidine decarboxylase.

BOVET and STAUB (1937) were the first to describe the protective properties of phenolic ethers which prevented the combination of histamine with cellular receptors. Thus, the antihistamine research with structure-activity relationship started.

One of the most significant recent contributions to the study of histamine is that of RILEY and WEST (1953) who found that there is a direct proportional relationship between the histamine content and the mast cell count of tissues.

As already pointed out, a new concept on the possible physiological role of histamine, has been put forward by KAHLSON (1960), who suggested that the histamine forming capacity of rat tissues is related to tissue growth.

B. Distribution and Location of Histamine.

BEST, DALE, DUDLEY, and THORPE, (1927) were the first to show that histamine is a normal constituent of a variety of organs. Since then through the collective work of many other investigators it has been amply confirmed that this substance is very widely distributed in the body, to such an extent that there is scarcely any organ which does not contain some histamine.

A puzzling fact about the distribution of histamine is that its content for the same organ varies in different species. For instance, in the liver of rats the average histamine content is less than 1 µg/gm. whereas in dogs the values range from 8 to 110 µg/gm. Histamine values also show considerable variations in the same organ within the same species (FELDBERG, 1956).

The presence of histamine has also been detected in many

physiological and pathological body fluids such as blood, plasma, bile, gastric juice, urine, nasal secretion, blister fluid and pus.

Histamine also appears in plants such as nettles, spinach, and others. These findings are mostly attributed to BEST and McHENRY (1931) and to BARGER and DALE (1910a), who also isolated histamine from ergot.

Regarding the location of histamine, RILEY (1953) has contributed greatly in this field. He provided evidence that the mast cell content and the histamine content of a tissue are closely correlated. The mast cells were discovered by EHRLICH in 1879, and since then their distribution and properties have been studied. Their function, however, still remains a matter of speculation. In mammals the mast cells occur mostly in the skin, the subcutaneous connective tissue, the mesentery, the lungs and the pleura. RILEY and WEST (1955b and c), showed by the use of histamine liberators, that the mast cell content of tissues is also reduced in parallel with the loss of histamine. This direct relationship of mast cell count and histamine content in the tissue was confirmed by the findings of GRAHAM and colleagues (1953, 1955) who used the technique of serial sections from frozen tissue. More recently RILEY (1959) also found that mast cells accumulate around growing tumors.

Although all the above studies established that the mast cell is a sort of store-house of histamine and the bulk of histamine present in the tissues is in the mast cells, it has been proposed by FELDBERG and TALESNIK (1953) and FELDBERG and MILES (1953) that some of the histamine must be in other sites as well. This is indicated since for example, the pyloric stomach has a high content of histamine but a low number of mast cells.

Inside the cells histamine is contained in intracellular particles. HAGEN (1954) demonstrated by differential centrifugation of homogenized dog liver that most of the intracellular histamine was located in the large granule fraction.

Further studies by HAGEN, BARRNETT and LEE (1959) on the intracellular distribution of histamine in mastocytoma cells indicated that histamine and heparin are stored within the basophilic granules. Metachromatic basophilic granules are found both in the basophil granulocytes of the blood and the tissue mast cells. This led to speculation as to whether both cell types have a common function and origin. There are indications that this might be so. The work of SHELLY and JUHLIN (1961), for example, suggests a participation of blood basophils in hypersensitivity reactions.

Finally, it must be mentioned that the recent literature

is lacking in giving any decisive opinion regarding the mode of binding of histamine. A concrete indication of the binding sites, the arrangement of unknown nature that holds intracellularly formed histamine, would be a major advancement in the research of this active substance.

C. Formation and Inactivation of Histamine.

Formation.

Histamine is present in most mammalian tissues, but its mode of formation is still not clear. According to BLASCHKO (1945) there are two main theories:-

i) Histamine is a vitamin, formed outside the body by bacterial decarboxylation of dietary histidine in the alimentary tract;

ii) Histamine is a metabolite, formed from circulating histidine by the histidine decarboxylase present in some tissues of the body.

That bacteria form histamine by decarboxylation of histidine is known for a long time (MELLANBY and TWORT, 1912). The occurrence of a mammalian histidine decarboxylase was first demonstrated by WERLE (1936). WILSON (1954a, 1954b) obtained evidence in the rat that part of the bacterially-formed histamine was absorbed, and accounted for part of the amine content found in the mucosa of the small intestine. He showed, that when the bacterial flora of the intestine was

inhibited by orally administered antibiotics, the urinary excretion of histamine decreased. He deduced, that part of the histamine bound in body tissues is absorbed from the intestine. WATON (1956) developed an improved technique for the estimation of mammalian histidine decarboxylase activity. There was no evidence for this enzyme in any tissue of cats and dogs, and he suggested that the carnivorous animals obtain their requirement of histamine from an exogenous source, like a vitamin, whilst the rodents make their own. On the other hand, SCHAYER (1952) using radioactive tracer techniques found that radioactive ^{14}C histamine was not taken up by the tissues but was detected in the urine. He emphasised, that according to his results, exogenous histamine is not bound by tissues. SCHAYER (1960) now postulates that there are at least two types of histidine decarboxylases, one in mast cells to produce histamine which is immediately bound to the cell constituents, and the second of unknown anatomical location, producing free histamine. In a recent paper KAHLSON, ROSENGREN and THUNBERG (1963) also state that "all the histamine held by tissue cells was formed therein".

Inactivation.

Regarding the inactivation of histamine, studies indicate that there are four main mechanisms:-

(a) oxidation;

(b) acetylation;

(c) methylation of the ring;

(d) methylation of the amino group.

These main pathways are illustrated in Fig.(1).

(a) Oxidation.

DALE and LAIDLAW (1911) produced evidence that animal tissues are capable of inactivating histamine, and EUSTIS (1915) described the enzymatic destruction of histamine.

BEST and McHENRY (1930) illustrated the inactivation of histamine by different animal tissues. At that time the term "histaminase" was suggested, since the reaction was considered to involve the specific enzymatic oxidative deamination of histamine.

The term "diamine oxidase" was introduced later by ZELLER (1938) who indicated that the enzyme also degrades putrescine, cadaverine and agmatine. KAPELLER-ADLER (1956) has questioned this terminology, since she believes that the two enzymes are not identical.

The in vivo studies of MEHLER, TABOR and BAUER (1952); TABOR, MEHLER and SCHAYER (1953); BOUTHILLIER and GOLDNER (1953) confirm that the degradation of histamine leads to imidazoleacetic acid which can be recovered from the urine of rats after injections of histamine. This was first

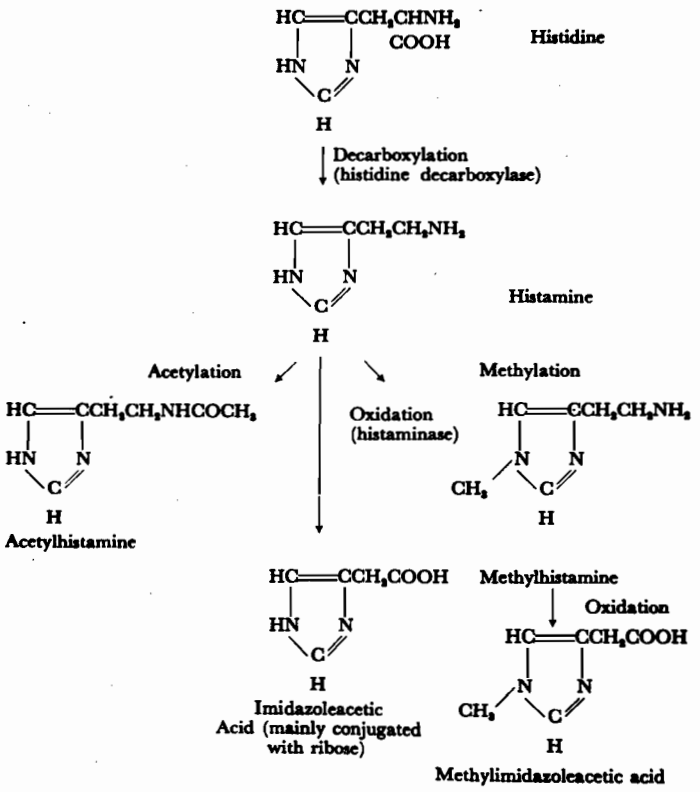

Fig. 1. Main pathways of histamine metabolism.

illustrated *in vitro* by ZELLER (1951), who showed that oxidation of histamine by diamine oxidase resulted in the formation of imidazolealdehyde which is then oxidized to imidazoleacetic acid (TABOR, 1951).

(b) Acetylation.

Histamine is excreted in most species in an inactive conjugated form. ANREP et al. (1944) described that in man considerable quantities of histamine are acetylated and are present in the urine as acetylhistamine. SCHAYER (1956c) found that only small amounts of histamine are acetylated in the tissues and that the recovered conjugated histamine is formed by bacteria in the intestine.

(c) Methylation of the ring.

SCHAYER (1959) demonstrated a pathway of histamine inactivation that involves two enzymes, whose identification has not yet been accomplished. Firstly histamine is methylated to methylhistamine which is then oxidized to methylimidazoleacetic acid. This compound has been identified in the urine, as well as some of the methylhistamine.

(d) Methylation of the amino group.

This pathway has been referred to by GADDUM (1962) but very little work has been done on this as yet.

It should be noted that the pathways of histamine inactivation vary in different species, sex, and also according

to the route of administration of histamine.

D. Pharmacological Actions of Histamine.

(a) Effect of histamine on gastric secretion.

Histamine's powerful stimulatory effect on the secretion of alimentary juices was first described by DALE and LAIDLAW (1911). Since then various studies have been performed in different species in order to find an explanation of the mechanism of this action. BEST and McHENRY (1930) have indicated the absence of the histamine destroying enzyme (diamine oxidase) from the gastric mucosa. FELDBERG and HOLMES (1941) observed that injection of histamine releasers led to an increase in secretion of acid gastric juice. Finally, it has been concluded by CODE (1956) that histamine is the final common local chemostimulator of the parietal cell of the gastric mucosa.

(b) The action of histamine on the skin.

When histamine is injected intradermally in minute doses or released from damaged human skin it induces a series of phenomena called by LEWIS (1927) the "triple response". Firstly, there is reddening of the skin caused by capillary dilatation; secondly, there is a formation of a weal due to exudation of plasma protein and fluid through the capillary walls into the extracellular space; and lastly, a flare appears which is due to dilatation of the arterioles, since histamine acts as a chemical stimulus at the origin of an axon

reflex. The triple response of human skin also occurs in some animal species.

ROSENTHAL and MINARD (1939) were the first to consider that skin histamine may be a peripheral mediator of pain. BROADBENT (1953) has attributed the itching sensation that is produced by cowhage to the presence of a histamine liberator. FELDBERG (1954) has speculated that histamine, when released in minute quantities near the sensory nerve endings in the superficial layers of the epidermis, may be the cause of itching without being able to produce the triple response.

(c) The action of histamine on smooth muscle.

Histamine induces contraction of nearly all smooth muscle. The muscles of the uterus, intestine and brochioles are the most sensitive to histamine but this sensitivity is somewhat dependant on the species.

FELDBERG and SMITH (1954) have proposed that histamine plays a role in the intestinal motility. However, this is not clearly decided due to the fact that there are many other smooth muscle stimulating substances in the wall of the digestive tract. These substances may take part in the intestine's motor activity, especially in those species which are not so sensitive to histamine.

(d) The effect of histamine on the vascular system.

When histamine is injected into the general circulation in small amounts a transient fall in arterial pressure can be

recorded in most species, an effect which is due to relaxation of the arterioles and capillaries. DALE and RICHARDS (1918) observed that larger vessels undergo a constricting action. Small doses of histamine increase the heart rate and strengthen the contraction while large doses may have the opposite effect. It has been proposed by KLISIECKI and HODBUT (1937) that the action of histamine on the heart may be the primary cause of histamine shock. LEWIS (1927) suggested that reactive hyperaemia which follows a circulatory arrest is due to the liberation of histamine. This was also confirmed by ANREP and his colleagues (1936, 1939, 1944) who found an increase in the histamine content of the effluent blood from an organ or limb during the hyperaemic state.

SCHAYER believes that the microcirculatory system is regulated by newly formed free histamine, which is synthesized in an amount that is required to satisfy the local needs for blood (SCHAYER, 1962).

That histamine is a mediator of various vasodilator phenomena, however, has not been confirmed by EMMELIN and EMMELIN (1947) and others.

WHELAN (1956) studied the relationship of histamine to the control of the peripheral circulation in man and was unable to demonstrate that histamine played any part in vasodilatation produced by various factors such as brief periods of circulatory arrest, adrenaline infusion, or exposure of the

hand or fingers to cold. He also observed that the vasodilation was not affected by the antihistamines.

(e) The role of histamine in anaphylaxis.

DALE and LAIDLAW (1910) observed the similarities that exist between the immediate symptoms of anaphylactic shock and those elicited by a large dose of histamine. Since then many studies have demonstrated in vivo, as well as in vitro, the release of histamine from sensitized organs into the blood or perfusion fluid after the administration of antigen. Evidence also indicates that histamine is released in many types of human allergy. However, it is not certain as to whether histamine is related to all allergic and hypersensitive reactions.

(f) Role of histamine in inflammatory reactions.

In acute inflammation there is a temporary disappearance of the mast cells in the affected area. As the inflammation subsides there is a gradual increase in the number of mast cells. These findings were confirmed by many authors (BENSLEY, 1950; McGOVERN, 1957). After the healing is completed the local hyperaemia subsides and the fibrous tissue becomes progressively less cellular, and finally dry and sclerotic. As this process proceeds the local mast cell number likewise decreases. The observations of ASBOE-HANSEN (1950) and NUMERS (1953) indicate some active relationship between the mast cell and its constituents, and the processes of wound healing, inflammation and repair.

(g) Effect of histamine on growth.

FELDBERG and LOESER (1954) have indicated that the advanced malignant growth is associated with decreased histamine content of the skin.

KAHLSON, ROSENGREN and WHITE (1958) investigated the source of increased histamine formation in the pregnant rat and found it to be in the foetal liver. When histamine formation was inhibited artificially by feeding a histidine and pyridoxine-deficient diet with parenteral administration of semicarbazide, the foetal development was interrupted (KAHLSON and ROSENGREN, 1959a and 1959b). Studies on other types of growth indicated that reparatively-growing tissues in skin wounds of the rat are accompanied by increased histidine decarboxylase activity, leading to an increased histamine formation. The rate of healing was retarded by lowering the histamine forming capacity, and it was accelerated by artificially increasing the histidine decarboxylase activity (KAHLSON, NILSSON, ROSENGREN and ZEDERFELDT, 1960). BOYD and SMITH (1959) found that depletion of skin histamine retarded wound healing and administration of histamine advanced the rate of healing.

The experimental findings of KAHLSON seem to indicate that histamine plays an important role in processes concerned with tissue growth. KAMESWARAN and WEST (1962), however, reported results which are contradictory to this theory and state, that "the role of an increased histamine forming capacity of a tissue is not directly related to growth or regeneration or repair".

(h) <u>Histamine as humoral transmitter</u>.

While histamine can be extracted from many nerves there is no evidence that histamine is accumulated in the nerve endings and released in vivo following nerve stimulation. The concept of histaminergic nerves has not been substantiated by experimental findings. The literature on this subject has been reviewed by PARROT (1954), and EULER (1956).

(i) Hormonal influence on tissue histamine content.

Adrenal cortical steroids influence the histamine content of tissues in the rat. It is increased after adrenalectomy and reduced after injection of cortical steroids (ROSE and BROWNE (1941), MARSHALL (1943), HICKS and WEST (1958a, 1958b), BARTLETT and LOCKETT (1959), TELFORD and WEST (1960). SCHAYER and his colleagues (1955) demonstrated that cortisone inhibits the binding of newly-formed histamine in rat tissues.

FELDBERG and LOESER (1954) estimated the histamine content of human skin in different clinical disorders and showed, that it was increased in hyperthyroidism or after the administration of thyroid hormone, but decreased in hypothyroidism.

E. Histamine - Releasing Agents.

Many non-specific chemical agents such as alkalies, mineral acids, organic solvents, etc. may lead to histamine release. The action of these substances has been well known since the studies of LEWIS (1927); it is considered to be a consequence of general injury to cells. The substances to be discussed below are considered to be more specific; their pharmacological actions are accounted for in large part by the histamine they release.

Peptone was the first histamine releaser to be discovered. Hydrolyzed protein in the form of Witte's peptone produces in the dog effects similar to anaphylaxis. In 1937 FELDBERG and O'CONNOR noted that perfusion of cat and guinea-pig lungs with solutions containing peptone were capable of releasing 10% and 3% respectively of the histamine content of this particular tissue. At about the same time DRAGSTEDT and MEAD (1937), and WILANDER (1938) indicated that in the dog both histamine and heparin are released in peptone shock. Since then many compounds have been shown to have a histamine releasing property. These compounds can be grouped as follows:-

(a) Histamine releasers of basic nature.

Studies of MacINTOSH and PATON (1949) on compounds such as diamines, diamidines, diguanidines, diisothioureas, diquaternary salts, polylysine, and those of PATON (1951) on compound 48/80

led to certain observations that can be summarized in a general way. The only common feature of these compounds from a structural point of view is the presence of one or more basic nitrogen atoms. It seems, that any compound containing basic groups that are separated by more than 5 or 6 atoms have the possibility of being a histamine releaser. Of the monobasic compounds the most active ones have their basic groups attached to substituted aromatic rings, like compound L1935 (a substituted butylamine, trimetaphan, morphine, and the substituted benzamidines. (FELDBERG and LECOMTE, 1955).

Compound 48/80 is a polymer of N-methyl-p-methoxy-phenylethylamine. PATON (1951) provided evidence that this compound is a potent histamine liberator. Intravenous injection in the cat resulted in marked delayed depressor response at doses of 0.5 - 1 mg/kg. FELDBERG and PATON (1951) reported that 48/80 in the isolated cat skin resulted in the appearance of 10 - 100 molecules of histamine in the perfusate per molecule of 48/80 injected. The rat is also sensitive to the actions of 48/80, other species being more resistant.

Although histamine release can account for all the effects of 48/80 ordinarily seen, it has other actions as well, such as a ganglion-blocking action (PATON, 1957), and a moderate antibacterial action (NORTON and de BEER, 1955). Neither of these actions modify the view, that in moderate doses the action

of 48/80 is that of histamine release (PATON, 1957).

(b) Centrally active substances as histamine releasers.

The release of histamine from skin and muscle in the cat by opium alkaloids has been demonstrated by FELDBERG and PATON (1951). It was found that centrally active substances such as apomorphine, codeine, morphine, nalorphine, and thebaine may release histamine to such an extent as to provide an explanation for their side effects as described by FELDBERG and his colleagues (1950), such as lethality in bronchial asthma, urticarial responses, and anaphylactoid reactions. SCHILD and GREGORY (1947) described histamine liberation by strychnine. The administration of hydralazine may also lead to histamine release.

(c) Antibiotics and chemotherapeutic agents as histamine releasers.

ORMEROD (1951) has shown that the polybasic trypanocidal drug antrycide has histamine releasing properties. In the field of antibiotics the agents which have histamine releasing properties are as follows: chlortetracycline, licheniformin and polymyxin B and E. This has been indicated in the work of MacINTOSH and PATON (1949) and also of NORTON and de BEER (1955).

(d) Histamine releasers of high molecular weight.

Egg-white and ovomucoid have been reported to release histamine. Many authors have contributed to this study, amongst others are LEGER and MASSON (1948) and HALPERN and BRIOT (1950).

Horse serum has also been reported to have histamine releasing properties (FELDBERG and SCHACHTER, 1952). Other substances in the same group are dextran, polyvinylpyrrolidone, and anaphylatoxin.

(e) Muscle relaxants and their histamine releasing property.

All the muscle relaxants appear to have a histamine releasing property. However, the potency varies among these compounds and the order of activity from the most to the least active is as follows: d-tubocurarine and its methyl ether, laudexium, mytolon, decamethonium, succinylcholine, and gallamine. In clinical practice it is only with d-tubocurarine that the histamine releasing activity could be expected.

(f) Sympathomimetic amines as histamine releasers.

It has been known for some time (ERLANGER and GASSER, 1919) that adrenaline can produce a shock-like state both in man and in animals. FREEMAN, FREEMAN, and MILLER, (1941) noted that sustained infusions in the dog led to increased capillary permeability, a fall in plasma volume, shock and haemorrhages in the endocardium and duodenal mucosa. Whether these actions are at least partly due to adrenaline is a matter of controversy. Sympathomimetic amines that are proven to release histamine from isolated cat-skin preparations are: phenylethylamine, tyramine and amphetamine but not ephedrine (PATON, 1957).

(g) Miscellaneous histamine releasing agents.

It has been found by ELDRIDGE and PATON (1954) that the amino acids,arginine and lysine, with their derivatives, ornithine and carnosine, are histamine releasers but of low potency, about 1/10,000 that of 48/80. Gastrin releases histamine from perfused muscle or perfused skin. (SMITH, 1954). Its histamine releasing effect from the stomach mucosa is relatively slight and probably insufficient to account for the full secretagogue effect of gastrin on the stomach.

Other histamine releasing substances in the literature are 5-hydroxytryptamine, tryptamine, leucotaxin, lymphagogues, thalassine and cowhage. ARUNLAKSHANA (1953) has reported that various antihistamines have a histamine releasing property.

It should be noted that histamine-releasing agents are of different potency. Their ability to liberate histamine also varies according to species, organs, and mode of application. An account of this is given throughout the literature, and it has been summarized by PATON (1957) who also gives an account of other substances that are liable to be released by the use of various histamine liberators, such as heparin, slow reacting substance and 5-hydroxytryptamine. Refractoriness to repeated doses of histamine liberators has been noticed throughout their study. FELDBERG and TALESNIK have attributed this phenomenon to the exhaustion of the releasable histamine. RILEY and WEST (1955) have noticed that after continuous administration of

48/80 the histamine content of rat subcutaneous tissue gradually increases again. This is accompanied by the appearance of small dense new mast cells which now resist the degranulating action of the histamine liberator. This process suggests that a full and sustained histamine depletion of any tissue will be difficult to achieve. Another factor that may contribute to the refractoriness to these compounds is that heparin may form an inactive complex with the histamine liberator. The mechanism by which these compounds liberate histamine is not known. It is believed that no single mechanism can account for all types of histamine release, even the same compound may bring about this action by different mechanisms. Thus, compound 48/80 acts on guinea-pig tissues as a single organic base probably by ion exchange (MONGAR, 1957), but its action on rat tissue involves an enzymatic mechanism (HOGBERG and UVNÄS, 1960). It is known that some releasers are able to act on mast cell granules (COPENHAUER, NAGLER and GOTH, 1953) and others require intact cells, as has been noted by MONGAR and SHILD (1956). LAGUNOFF and BENDITT (1960) have demonstrated histamine releasers which are active only in the whole animal. This probably suggests an activating mechanism that is present only <u>in vivo</u>.

Thus it seems proper to say that the main difficulty in these studies is lack of understanding the manner by which histamine is bound to its site.

F. Principal Methods of Histamine Estimation.

All methods described below are given in Methods of Biochemical Analysis Vol.III (1956)(Interscience Publishers, New York).

1. Biological Assay.

(a) Blood pressure assay.

This method was introduced by BURN (1928). It is based on the prompt fall of blood pressure that is seen in dogs and cats after intravenous injection of histamine.

The animal is anaesthetized usually with chloralose (80 - 100 mg/kg) or pentobarbital sodium (25 - 30 mg/kg) given intravenously. The level of anaesthesia should be kept constant throughout the assay, since change in the depth of anaesthesia alters the sensitivity of the preparation to histamine.

Apparently the extracts prepared from blood or tissue often contain some cholinergic activity. As a rule therefore the cat is atropinized intravenously with 1-2 mg. of atropine sulfate before the assay, for more accurate histamine determination.

The blood pressure is recorded from the carotid or femoral artery and the injections are given through a siliconized glass cannula or polythene tube tied in a systemic vein. The cannula or tubing is connected to a buret from which 2 or 3 ml. of physiological saline solution is allowed to wash each injection.

The injections are made via a needle, or a T tube that is placed in the tubing close to the vein.

The extent of the blood pressure fall is proportional to the amount of histamine injected and provided the dose is not too large, the recovery of the blood pressure is complete and the response can be obtained repeatedly without loss of sensitivity. Usually a dose of 0.5 to 1 µg of histamine will give a satisfactory fall of blood pressure. Once the standard dose is decided the injections are given at definite intervals (2 or 3 minutes).

Some preliminary test-injections of the unknown solution will be required in order to gain information about its approximate strength. The aim is to give that amount of the unknown solution which matches the decline of blood pressure which is produced by the standard. The histamine concentration of the unknown solution can than be calculated.

(b) Assay of Histamine on the guinea-pig ileum preparation.

This method for the quantitative estimation of histamine was used throughout this study. For its detailed description see: EXPERIMENTAL WORK: METHODS.

2. Chemical Assay.

(a) Azo-dye Method.

This method was introduced by ROSENTHAL and TABOR (1948) and it depends on the coupling of histamine with a diazotized aromatic amine (p-nitro-aniline) to produce an azo-dye which after some purification can be measured spectrophotometrically. The position of coupling on the histamine molecule is not known and it is suspected that the coloured product may be a mixture of derivatives. This method has three main disadvantages. Firstly, the product deteriorates about 7% in the first hour; secondly, this method requires five times the minimal amount of histamine that can be measured by the DNFB method, which will be mentioned below; and thirdly, it is believed that other substances found in tissue extracts may interfere with the estimation of histamine.

(b) 2,-4-Dinitrofluorobenzene Method.

This method for the quantitative estimation of histamine was introduced by McINTIRE et al (1950). Histamine reacts with 2, 4 dinitrofluorobenzene (DNFB) to form two different derivatives: Derivative I, which is Nα -(2,4 dinitrophenyl) histamine and Derivative II, which is Nα ,N$^{\prime}$ -bis (2,4 dinitro-

phenyl) histamine. It is possible to obtain exclusively Derivative I by controlling conditions such as pH and concentration of DNFB. Derivative I is a yellow compound with an intense light-absorption maximum at 355-360 mμ. The optical density of this solution at λ 358 mμ is a measure of the amount of histamine present. Before measuring, the solution is prepared by removal of the excess dinitrophenol, and of the dinitrophenyl derivatives of the monoamines, diamines, and polyamines.

With this method one can determine histamine in tissue extracts with an error of± 5-7%.

(c) Isotope-dilution Method.

This method was introduced by SCHAYER et al (1955) and is based on the reaction of histamine with I^{131} - labeled p-iodosulfonyl (pipsyl) chloride to give labeled dipipsyl histamine. This is mixed with a large excess of non-labeled carrier dipipsyl histamine which is then recrystallized to constant specific activity. Different solvents are used for successive crystallizations, and treatments with different activated charcoals are applied.

This method is the most specific, the most sensitive but the most time-consuming of the chemical methods.

(d) Fluorometric Method.

This method was recently introduced by SHORE (1959) (see reference) and involves extraction of histamine into butanol from alkalinized perchloric acid tissue extracts, return to an aqueous phase, and condensation with o-phthalaldehyde to form a fluorescent product which is estimated in the spectrofluorometer. Histamine can be measured in as low a concentration as 0.005 µg/ml.

II EXPERIMENTAL WORK

Materials and Methods

A. Materials

1) All glassware was cleaned with acid dichromate mixture or detergent (Sparkleen from Fisher Co.) and used only after thorough rinsing and drying.

2) The ordinary inorganic salts, organic solvent, etc. were Merck Reagent grade or its equivalent. Trichloroacetic acid (100% $^{w}/_{v}$ sol.) was purchased from Fisher Scientific Co.

3) Agents used for animal treatments:-

a)	Compound 48/80.	Obtained from the Wellcome Research Laboratories.
b)	Cortisone acetate.	"Cortone" injections, obtained through the courtesy of Merck, Sharp and Dohme Ltd.
c)	Octylamine.	Supplied by the Eastman Org.Chemicals.
d)	Acetylsalicylic acid, Sodium salicylate	Supplied by Lymans Ltd.

4) Animals.

Two strains of guinea-pigs have been used. An albino strain from a closed colony was obtained from the Canadian Breeding Laboratories and a multicoloured short haired variety from the Quebec Breeders Association. For the determination of normal histamine values, guinea-pigs

weighing 300-400 gm. of both sexes, from both strains were used. When it was established that a sex difference in histamine contents of guinea-pig organs exists, subsequent studies were performed on "ordinary" non-albino male guinea-pigs weighing 300-400 gm., obtained from the Quebec Breeders Association. Guinea-pigs were fed with a diet of Purina Guinea-pig Chow, water and hay ad libitum. Three animals were kept in each cage and all guinea-pigs observed for one week before being used.

B. Methods.

1) Control injections.

Agents used for various treatments were dissolved in physiological saline or distilled water. Control injections with these solvents were always given via the same route, for the same time interval as the agent in question. Control values in the case of physiological saline did not differ from normal values obtained from untreated animals. Injections with distilled water, however, caused an approximately 52% increase in lung histamine values, compared to untreated controls. Although making use of these data would have offered an advantage in the depletion experiments, it was considered that normal tissue histamine levels of guinea-pigs are those obtained from normal, uninjected animals. Therefore it was decided to compare experimental data with data obtained from

untreated guinea-pigs, which are used as "control values" throughout the study.

2) Method of extracting histamine from tissues.

For histamine extractions the modified CODE (1956) method, as well as one similar to that described by RILEY and WEST (1953) were initially used. Since the two methods were in agreement, the modified RILEY-WEST method was selected for routine use.

Procedure.

The guinea-pig was killed by a blow on the head, exsanguinated and the organs immediately removed. Histamine content of the lungs, skin of external ear, small intestine, heart, liver, kidney and spleen was determined at the beginning of these studies. Due, however, to the time-consuming procedures of extraction and biological assay it often became necessary in the course of the work to reduce the number of organs to be tested. The lungs were first removed from each animal, washed immediately, dried on filter paper and weighed. Lungs exceeding 0.8% of the body weight were considered pathological and such animals were not used. The other organs were placed separately into physiological saline solution. All these organs were prepared for extraction (dried and weighed) within one hour of their removal from the body.

Corresponding organs of 2 or 3 guinea-pigs were pooled, taking equal weights of each organ from each animal. The tissue was then cut into fine pieces with two scalpels and placed

in the homogenizing flask, together with 2 ml. of 10% trichloroacetic acid (TCA) per each gram of wet tissue. (Water or saline solution should not be added at this point since the dilution of the 10% TCA will render the precipitation of proteins incomplete). The tissue was homogenized with a Virtis 45 homogenizer for 15 minutes at the speed of 45,000 r.p.m. At the end of this time the flask was taken off and sprinkled with as little distilled water as possible to wash the blade of the homogenizer. The tissue homogenate was carefully transported into the centrifuge tubes and placed in a Servall angle centrifuge which was then rotated for 15 minutes at 6,000 r.p.m. After centrifugation the clear supernatant was decanted and extracted with 20 ml. of ether in order to remove fat and excess TCA. The aqueous layer which contained the extracted histamine was withdrawn into a 250 ml. beaker and the ether was washed with 10 ml. of distilled water in order to remove any histamine which might have been taken up by the ether. A few minutes later the water was withdrawn and added to the first aqueous layer in the beaker. The content of the beaker was heated over a Bunsen flame in order to evaporate the traces of ether that were carried into it. After 5 minutes of boiling the extract was allowed to cool down. At this stage the extract could be stored in the freezer or tested. Immediately before the biological assay the extract was brought to a pH of 7.2-7.5 from pH 2.0-2.5 by the addition of 1N NaOH. The volume

of the extract was sometimes increased with distilled water in order to suit the needs of the bioassay. (see below).

3) Quantitative method for testing the extracted histamine (Biological Assay).

The histamine extracted from the selected tissue was quantitatively tested on the isolated guinea-pig ileum preparation. This method was introduced by GUGGENHEIM and LOEFFLER (1916) and since then it has been used successfully by many workers. It is believed to be reliable regarding sensitivity and accuracy.

a) Physiological solution. (Tyrode solution)

The solution was made up freshly before each use. Ten liters were prepared at a time, as follows:

A bottle of 10 liters capacity was filled with distilled water up to the 9 liter mark. The following agents were added (one at a time followed by a thorough mixing) in the order described below:

1. 80 gm. NaCl
2. 10 ml. of 5% NaH_2PO_4 solution
3. 20 ml. of 10% KCl solution
4. 5 ml. of 40% $MgCl_2$ solution
5. 10 gm. $NaHCO_3$ (dissolved separately)
6. 10 gm. glucose
7. 10 ml. of 20% $CaCl_2$ (anhydrous) solution
8. 5 ml. of 0.1% atropine sulfate solution
9. Distilled water to make up 10 liters

All solutions described on the previous page were made up as stock solutions and stored in the refrigerator. The concentration of atropine sulfate was 5×10^{-7} and it was added to the Tyrode solution in order to eliminate or reduce cholinergic activity.

b) Apparatus.

A plastic 6 x 6 x 6 inch water bath was arranged as shown in Figure (2). The organ bath had a capacity of 15 ml. The temperature of the water bath was maintained at 35°C by means of a thermostat and a heating element that were both placed in the water bath. As can be seen from the diagram the Tyrode solution entered the system from the reservoir through the opening of a stop-cock. Before entering the organ bath the solution passed through a coil which helped to maintain a constant temperature of the solution. By opening a valve placed on the outlet tube the Tyrode solution could be drained away from the organ bath.

A thin glass tube was bent in such a way as shown in the diagram and placed in the organ bath. This tube provided the inlet of the mixture of 5% CO_2 and 95% O_2 which was continuously supplied to the physiological solution at the speed of 60 - 70 bubbles per minute. A platinum hook on the oxygen inlet tube, which was suspended above the bath, provided a means for attaching the gut within the bath. One thread attached the gut to this hook and another thread fixed the upper end of the gut to the

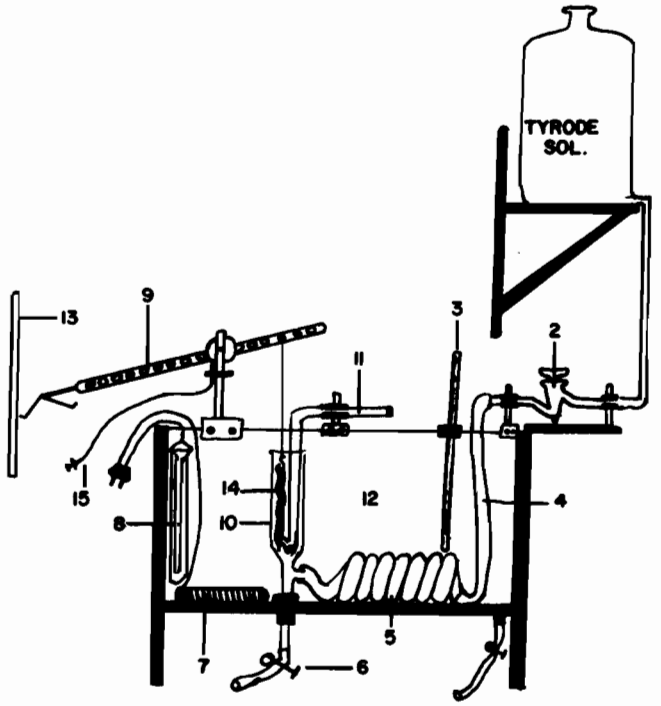

Fig. 2. Diagram of the apparatus used for the bioassay of histamine.

1. Reservoir for Tyrode sol.
2. Stopcock.
3. Thermometer.
4. Plastic tubing.
5. Glass coil.
6. Outlet.
7. Heating element.
8. Thermostat.
9. Lever.
10. Organ bath.
11. Oxygen inlet tube.
12. Water bath.
13. Kymograph.

writing lever located directly above it. Plasticine was used to balance the horizontal lever. This lever was equipped with a frontal writing point which produced almost perpendicular tracings on the slowly moving kymograph drum. The magnification was approximately 3:1. A cable-release device at the fulcrum of the lever permitted steadying it when the bath fluid was renewed.

c) Preparation of the ileum strip.

Guinea-pigs of either sex weighing 200 - 300 gm. were killed by a blow on the head and exsanguinated through the carotid arteries. The abdomen was opened and a piece of terminal ileum approximately 20 cm. long was removed and placed in a Petri dish containing freshly prepared Tyrode solution. The strip was gently washed with Tyrode solution be means of a 20 cc. pipette. A section 4 - 5 cm. long was cut and attached to the hook of the glass tube (mentioned above) by one end, and to the hook with the thread by the other end. Both ends of the gut remained open. The glass tube was then carefully placed in the organ bath, secured, and the thread fixed on the lever. At this point the lever was balanced by plasticine and the lever was adjusted for best efficiency. The oxygen carbon-dioxide mixture was turned on at the speed given above. The gut was then left to become accustomed to its new environment for

approximately 15-20 minutes after which time the bioassay started.

d) Preparation of the histamine standard.

Throughout this study histamine is expressed in terms of the base. The dihydrochloride was used as standard. The stock solution, which was always stored in the refrigerator for a period up to 20 days was made by weighing 16.5 mg. of the salt and dissolving it in a 100 ml. volumetric flask in distilled water. The stock solution therefore contained 100 µg/ml. of histamine in terms of base. From this concentration 0.5 ml. was pipetted into a 100 ml. cylinder and made up to 100 ml. by adding distilled water. This solution therefore had a concentration of 0.5 µg/ml. (5×10^{-7}) of histamine and was prepared freshly a few minutes before the bioassay.

In most cases a volume of 0.4 ml. (0.2 µg of histamine) of this solution introduced into the 15 ml. organ bath by means of a tuberculin syringe equipped with a #24 gage short needle was sufficient to produce a contraction of the ileum that was sensitive and reliable over small changes of histamine concentration. Always the same tuberculin syringe was used in the course of one experimental assay.

e) The Bioassay Procedure.

As previously mentioned, it was necessary at the start of the bioassay to find the dose of the standard histamine solution which gave a satisfactory contraction and over which small differences in the amount of histamine produced the greatest and most consistent changes in the length of the gut. Once this was decided the gut was stimulated to contract every two minutes. 1 ml. "tuberculin" syringes were used to introduce samples into the organ bath. Bath volume was maintained within $\pm$ 0.1 ml. The histamine was left in contact with the ileum for 20 seconds after which time the ileum was washed twice by filling, emptying and refilling the organ bath in the manner mentioned above (see part (b)). After the washing procedure the gut returned to its normal base line to be stimulated again at the next two minute interval. After a number of contractions the gut was standardized (equal amounts of histamine were giving equal contractile response). Having obtained three consecutive contractions of equal length on the smoked paper, the first dose of the extracted tissue-histamine was given. Since this dose was serving as an approximate indication of the histamine concentration of the extract, care was taken to give a very small amount. The tissue histamine dose was then washed as usual and the next response at the two minute interval was to be obtained by the standard solution. Injections with the standard solution were given until

the responses became of the same height as the ones that were obtained just before the tissue extract was given. Sometimes it was seen that three consecutive and equal responses were obtained that were somewhat different from the original contractions obtained with the histamine standard. These responses were used as controls for a new tissue-histamine dose. The aim was to match the heights of the contractions that were obtained by the standard with those obtained with the tissue extract. Once these responses were matched and could be repeated alternatively then the assumption was, that the amount of histamine present in a given volume of tissue extract was equal to the amount present in the standard. Since the concentration of the standard was known, the concentration of the tissue extract could be calculated by knowing the volumes and organ weights involved.

In some of the experiments exact matching of contractions caused by standard and unknown could not be established. In these cases two doses of the extract were considered, one which was a little higher and one which was a little lower than the response obtained with the standard. In such a case the average volume of the two values was used to calculate the total extracted tissue histamine (see Fig. 3).

The contractions of the gut induced by the extracted solution could be blocked by promethazine in concentrations of 10^{-8}, thus proving that they were caused by histamine and were not due to any other smooth muscle stimulating substance present in the extract.

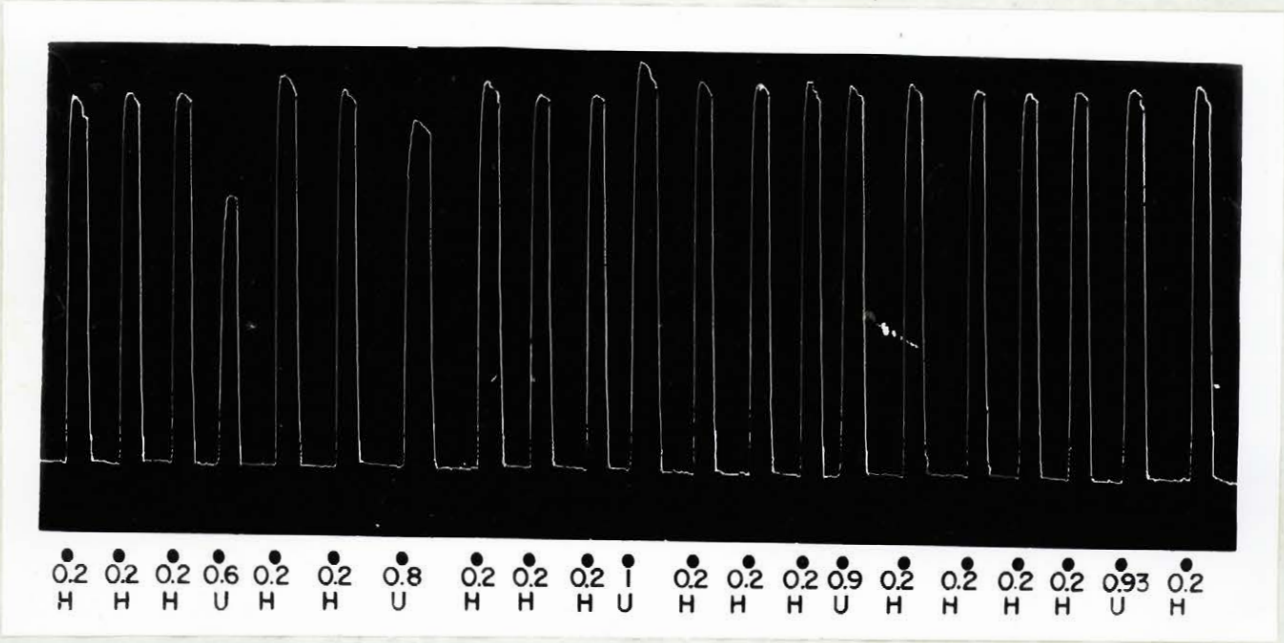

Fig. 3. Reproduction of a trace obtained during an assay of histamine on the guinea pig ileum preparation.

H = Histamine standard; figures in μg of histamine base.

U = Tissue extract prepared from guinea pig ear, expressed in ml.-s.

From this assay, 0.915 ml of U was considered to contain 0.2 μg of histamine.

C. Results.

1) Studies on untreated animals.

a) Normal values.

Individual variations in histamine contents are fairly great in different organs of individuals from the same species. In order to establish a firm base line for later work it was essential to determine normal values of a great number of animals. It was hoped to be able to obtain more uniform data if an appropriate strain of guinea-pigs could be used, and to this end a closed colony albino strain was employed. In each animal the histamine content of the lung, liver, kidney, small intestine, heart, skin of external ear and spleen was determined. Table I shows the average histamine values of the various organs in a group of 30 guinea-pigs, comprising both males and females.

Two observations were made in the course of these studies. It became apparent that the use of a closed colony guinea-pig strain does not offer any advantage as far as individual variations in histamine contents are concerned. Therefore, for technical reasons, it was decided to switch over to ordinary, non albino guinea-pigs, which were to be obtained always from the same breeder. Some differences were also apparent between tissue histamine content of males and females (see below), and for this reason it was also decided to use only male animals for all experiments. Control histamine values for subsequent studies are tabulated in Table II. These are values obtained from 12 male guinea-pigs, weighing 300-400 g.

TABLE I

Normal histamine content of guinea-pig organs, expressed as the base in µg/g tissue. Each group represents average histamine values of 3 albino guinea-pigs

Group	Lung	Ear	Gut	Liver	Kidney	Heart	Spleen
I	33.3	22.2	18.0	2.4	3.1	6.3	4.2
II	19.0	20.3	28.5	2.3	3.1	9.1	4.2
III	39.3	27.5	23.7	2.0	2.7	11.0	4.8
IV	27.7	21.3	24.4	1.9	3.2	7.4	3.7
V	20.8	20.2	27.1	2.7	2.4	8.4	3.9
VI	21.9	14.0	14.2	1.7	2.3	-	9.0
VII	19.8	20.7	16.0	1.7	3.2	-	6.7
VIII	23.3	25.3	21.3	2.2	3.4	6.5	6.8
IX	16.4	15.6	13.9	1.7	3.2	-	6.7
X	28.2	21.2	17.3	2.7	3.1	7.0	10.0
Average Value	25.0 ±2.1	20.9 ±1.2	20.6 ±1.6	2.2 ±0.1	2.9 ±0.1	7.6 ±0.6	6.2 ±0.7

TABLE II

AVERAGE TISSUE-HISTAMINE CONTENTS OF UNTREATED MALE GUINEA PIGS

Animals Per Group	Histamine content expressed as the base in μg/gm of fresh tissue						
	Ear	Lungs	Small Intestine	Liver	Spleen	Heart	Kidney
3	27.5	27.3	25.0	1.1	7.4	7.8	1.8
3	16.8	19.0	15.9	2.0	3.6	5.2	1.6
3	14.4	18.7	20.8	1.8	5.1	5.2	1.9
3	14.9	11.9	16.2	1.8	6.2	7.3	2.1
Mean:	18.4 ±2.7	19.2 +2.7	19.5 ±1.9	1.7 ±0.2	5.6 ±0.5	6.4 ±0.6	1.9 ±0.1

The other finding was, that the average histamine level of the female guinea-pig organs is higher than that of the male. (Groups I-V in Table I were obtained from female, groups VI-X from male animals). This basic finding has not previously been reported in the literature and it seemed worthwhile to test this point further.

b) Differences in histamine content in organs of female and male guinea-pigs.

Fifty-four guinea-pigs were used, twenty-seven males and twenty-seven females. Thirty animals were taken from an albino closed-colony strain and twenty-four others were ordinary short-haired non-albino guinea-pigs. Histamine contents of the lungs, small intestine, skin of external ear, heart, liver, kidney and spleen were determined. In both strains the average histamine content of all organs examined was higher in female than in male animals with the exception of the spleen. It can be seen from Table III that the average histamine values obtained from 27 female lungs, small intestines, ears, hearts, livers and kidneys are 11 - 33% higher than the corresponding figures for male organs. The result on the spleen histamine contents will be discussed subsequently.

One of the organs with the highest histamine content in the guinea-pig is the lung. As shown in Table IV, it was found

TABLE III

AVERAGE HISTAMINE CONTENT OF NORMAL GUINEA-PIG ORGANS: DIFFERENCE IN HISTAMINE CONTENT BETWEEN MALE AND FEMALE ANIMALS

Sex, No. of Guinea-pigs	Histamine content expressed as the base, in µg/g tissue						
	Lung	Small Intestine	Skin of Ear	Heart	Liver	Kidney	Spleen
Males, 27 animals	20.7±1.6	17.8±1.2	18.8±1.5	6.5±0.4	1.8±0.1	2.3±0.2	6.9±0.7
Females, 27 animals	27.4±2.2	23.2±1.4	21.0±0.9	7.9±0.6	2.2±0.1	2.6±0.1	4.8±0.5
% Difference Female >	33.2%	28.1%	10.5%	18.8%	16.3%	15.7%	female <24.9%

TABLE IV

DIFFERENCE IN HISTAMINE CONTENT BETWEEN MALE AND FEMALE GUINEA-PIG LUNG AND SMALL INTESTINE

	LUNG			SMALL INTESTINE	
	Males	Females		Males	Females
30 Albino Guinea-pigs	21.9 16.4 19.8 28.2 23.3	33.3 39.3 20.8 27.7 19.0	30 Albino guinea-pigs	14.2 13.9 16.0 17.3 21.3	18.0 23.7 27.1 24.4 28.5
Mean:	21.9±1.7	28.0±3.4	Mean:	16.5±1.2	24.3±1.6
Difference:		>27.8%	Difference:		>43.8%
24 Non-albino guinea-pigs	27.3 19.0 18.7 11.9	30.6 25.8 30.8 19.0	24 Non-albino guinea-pigs	25.0 15.9 20.8 16.2	29.4 17.8 21.2 19.0
Mean:	19.2±2.7	26.6±2.4	Mean:	19.5±1.9	21.9±2.3
Difference:		>38.5%	Difference:		>12.3%
Overall Mean:	20.7±1.6	27.4±2.2	Overall Mean:	17.8±1.2	23.2±1.4
Difference:		>33.2%	Difference:		>28.1%
Significance:	P<0.02		Significance:	P< 0.02	

Each value represents an average of 3 guinea-pigs. Histamine is expressed as the base, in µg/g tissue.

that the average lung histamine content of female, non-albino guinea-pigs was 38.5% higher, that of female albino guinea-pigs 27.8% higher than lung histamine values of the male counterparts. Taking both albino and non-albino groups together, the female average is 33.2% higher than the male average. This difference is significant ($P < 0.02$).

Also tabulated in Table IV are histamine contents of the small intestine taken from both sexes of both strains. Albino females have been found to contain 43.8% higher histamine levels in the small intestine than albino males, the difference in the non-albino strain being less, 12.3%. The overall difference in the average histamine content between female and male small intestines is 28.1%, the difference is significant ($P < 0.02$).

The average histamine content of the ear, heart, liver, and kidney is also higher in female than in male guinea-pigs (Table I). Differences, however, are not statistically significant in these organs.

As can be seen in Table V, the only organ in which the overall average in histamine content was higher in male than in female guine-pigs was the spleen. The difference is 24.9% and is significant. Yet, as can also be seen, this difference comes solely from results obtained in the albino strain. Since the weights of the spleens were not different in the two strains and sexes, the higher average histamine content of the male

TABLE V

AVERAGE SPLEEN HISTAMINE CONTENTS OF MALE AND FEMALE GUINEA-PIGS OF TWO DIFFERENT STRAINS

Albino Guinea-pigs			Non-albino Guinea-pigs		
30 Animals	Males	Females	24 Animals	Males	Females
Mean:	8.1±0.7	4.2±0.2	Mean:	5.6±0.5	5.8±1.1
Significance	P<0.01				

	Males	Females
Overall Mean	6.9±0.7	4.8±0.5
Significance	P<0.01	

Histamine is expressed as the base, in µg/g tissue

spleen in the albino guinea-pigs used seems to be characteristic to that albino strain.

2) Experiments performed in order to reduce histamine levels of guinea-pig organs.

a) Studies with Compound 48/80.

Compound 48/80 is known to be one of the most potent histamine liberators in cats and rats. Though the guinea-pig is not considered to be a suitable animal for the demonstration of 48/80 action, it has been found, that in the skin and perfused lung the histamine content could be diminished by the use of this agent (PATON, 1957). Recently FRICK, HALPERN and LIACOPOULOS (1962) reported that chronic 48/80 treatment reduced the pulmonary histamine content of guinea-pigs.

Nine guinea-pigs were treated with 48/80 for three days. The compound was dissolved in physiological saline, 1 mg. in 1 ml. Each animal received intraperitoneally 1 ml. of this solution twice a day (one injection in the morning and one in the late afternoon). On the third day the animals received only one dose in the morning, since the tissues were to be extracted 24 hours after the last dose. This time interval was thought to be necessary in order to eliminate the possibility of 48/80 being extracted with the tissue histamine and thus interfering with the bioassay. On the first day of treatment it was observed, that all animals showed signs of histamine release 1 hour after 48/80 administration. They had difficulty in breathing and were unable to keep their equilibrium properly. These observations,

however, did not appear as intense on the second and third day of treatment. The histamine values were found to be not much different from those of the control values given in Table II.

Since it was observed that 48/80 had histamine releasing properties, but for some reason the tissue histamine content was not reduced, it was decided to treat the animals for a longer time, in a manner similar as described by FRICK, HALPERN and LIACOPOULOS (1962).

Nine guinea-pigs were used. They were injected with 48/80 (1 mg/ml.) intraperitoneally twice a day, starting with 200 µg, increasing this dose by 50 µg each time to 800 µg and continuing at this dose until the morning of the 14th day. On the 15th day the animals were sacrificed and their tissue histamine extracted.

The histamine content of ear, lung, and small intestine was examined. As it can be seen from Table VI the histamine content of the ear was reduced by 2.7%, that of the lung by 18.3% and that of the small intestine by 10.7% compared to the control values.

These values are not much different from the range of the control values. However, there is a slight reduction. It was then decided to repeat the above treatment with the difference that animals were not to be sacrificed until one week after the last injection of 48/80.

TABLE VI

Treatment: 48/80 for 2 weeks, given i.p. twice daily in increasing doses. (For details see text)

Animals per Group*	Histamine content of pooled guinea pig organs expressed as the base in µg/gm of fresh tissue		
	Ear	Lung	Small Intestine
2	22.1	15.6	17.0
2	16.5	19.2	18.7
2	15.1	12.4	16.6
Mean:	17.9 ±1.7	15.7 ±1.9	17.4 ±0.5
Control Mean:	18.4 ±2.7	19.2 ±2.7	19.5 ±1.9
% Difference Control >	2.7%	18.3%	10.7%

* 3 animals died during treatment

Nine animals were injected intraperitoneally with 48/80 twice a day, starting with 200 µg, increasing this dose by 50 µg each time to 800 µg and continuing at this dose until the morning of the 14th day. The animals were then left untreated for a week.

Seven days after the discontinuation of 48/80, the animals were sacrificed and their tissue-histamine extracted. When the tissue-histamine extractions were bioassayed the values obtained indicated the following extent of reduction: Ear, 40.2%; lungs, 20%; small intestine, 10.3%. These values and the individual figures obtained are shown in Table VII.

SCHAYER and his colleagues (1955) indicated that cortisone inhibits the binding of newly-formed histamine in rat skin. It was hoped therefore that it might interfere with histamine binding in guinea-pig organs as well. In the next series of experiments administration of compound 48/80 was therefore combined with cortisone treatment.

In 9 guinea-pigs the treatment with 48/80 was repeated, (i.e. the animals received 48/80 intraperitoneally twice daily, starting with 200 µg. increasing by 50 µg each time to 800 µg and continuing at this dose until the morning of the 14th day) and one hour after each dose of 48/80, 50 mg/kg. of cortisone acetate was injected intraperitoneally.

This time interval between the administration of the two

TABLE VII

Treatment: as in Table VI. Animals were sacrificed 7 days after last injection.

Animals per Group	Histamine content of pooled guinea pig organs expressed as the base in µg/gm of fresh tissue		
	Ear	Lung	Small Intestine
2	9.7	16.7	15.4
3	12.1	16.4	14.8
3	11.1	13.0	22.4
Mean:	11.0 ±0.6	15.4 ±1.0	17.5 ±2.0
Control Mean	18.4 ±2.7	19.2 ±2.7	19.5 ±1.9
% Difference Control>	40.2%	20.0%	10.3%

compounds was chosen, since it appeared from previous experiments that the most prominent signs of histamine release occurred approximately 1 hour after intraperitoneal 48/80 administration. The animals were sacrificed on the 15th day. As can be seen from Table VIII the histamine content of the ear was 19% lower than that of the control, that of the lung 36.5% lower, while histamine content of the small intestine remained unaltered.

It was of interest to know, whether this reduction in tissue histamine persisted over the period of 7 days. For this reason 6 animals were treated in the same manner as mentioned above, but this time the animals were not sacrificed on the 15th day after the start of the treatment. They were left without any treatment for 7 days. At the end of this period the animals were sacrificed and the histamine was extracted. The bioassay showed that the histamine contents were reduced much more than by any other method described so far. The lung histamine content was 45.1% lower than that of the control; the ear 35.9%; the small intestine 29.2%; the liver 29.4%; spleen 21.4%; heart 25%; and kidney 10.5% - Table IX.

TABLE VIII

Treatment: 48/80 for two weeks, given i.p. twice a day in increasing doses: after each dose of 48/80, 50 mg/kg cortisone acetate was administered i.p.

Animals per Group*	Histamine content of pooled guinea pig organs expressed as the base in µg/gm of fresh tissue		
	Ear	Lung	Small Intestine
3	11.8	11.9	18.5
2	16.7	13.3	21.4
3	16.2	11.4	20.6
Mean:	14.9 ±1.2	12.2 ±0.5	20.2 ±0.7
Control Mean:	18.4 ±2.7	19.2 ±2.7	19.5 ±1.9
% Difference Control >	19.0%	36.5%	--

* 1 animal died during treatment

TABLE IX

Treatment: As in Table VIII. The animals were sacrificed 7 days after last treatment

Animals per Group*	Histamine content of pooled guinea-pig organs expressed as the base in µg/gm of fresh tissue						
	Ear	Lung	Small Intestine	Liver	Spleen	Heart	Kidney
3	12.4	8.0	11.6	1.0	3.8	4.1	1.4
2	11.3	13.0	16.0	1.3	5.0	5.5	2.0
Mean:	11.8 ±0.4	10.5 ±1.8	13.8 ±1.6	1.2 ±0.1	4.4 ±0.4	4.8 ±0.5	1.7 ±0.2
Control Mean:	18.4 ±2.7	19.2 ±2.7	19.5 ±1.9	1.7 ±0.2	5.6 ±0.5	6.4 ±0.6	1.9 ±0.1
%Difference Control>	35.9%	45.1%	29.2%	29.4%	21.4%	25.0%	10.5%

* 1 animal died during treatment

b) Studies with Octylamine.

MONGAR and SCHILD (1953) measured quantitatively the histamine releasing activity of a series of monoalkylamines using minced guinea-pig lung. They reported that the C_8 to C_{12} compounds of this series were found to be considerably more active in releasing histamine from guinea-pig lungs than compound 48/80.

Fifteen guinea-pigs were treated with octylamine for ten days. The compound was taken up in distilled water in a concentration of 3.88 mg/ml, and 5 mg/kg. were injected subcutaneously twice a day (once in the morning and once in the late afternoon) until the morning of the tenth day. On the morning of the 11th day the animals were sacrificed and their tissue histamine extracted. The histamine content of ear, lung,and small intestine was examined. As it can be seen from Table X the histamine content of the ear was reduced by 28.8%, that of the lung by 34.9% and that of the small intestine by 18.0%.

The same treatment was repeated with another group of nine animals but at this time the animals were sacrificed one week after the last dose of octylamine. It was seen that the histamine content of the same organs had returned to its control value. In connection with the octylamine studies it was also seen in another group of nine animals that a dose of 1 mg/kg. (instead of the 5 mg/kg. dose) did not reduce the tissue histamine.

TABLE X

Treatment: Octylamine 5 mg/kg for 10 days given s.c. twice a day

Animals per Group*	Histamine content of pooled guinea pig organs expressed as the base in µg/gm of fresh tissue		
	Ear	Lung	Small Intestine
3	15.1	17.4	15.7
3	12.4	11.0	16.6
2	9.8	9.5	13.9
2	13.0	11.8	17.6
3	15.3	12.9	16.1
Mean:	13.1 ±0.9	12.5 ±1.2	16.0 ±0.5
Control Mean:	18.4 ±2.7	19.2 ±2.7	19.5 ±1.9
% Difference Control >	28.8%	34.9%	18.0%

* 2 animals died during treatment

It was of interest to observe the effect of combined treatment with octylamine and cortisone, since as it was seen previously 48/80 combined with cortisone produced a marked reduction in the histamine content of organs tested.

Nine animals were treated with octylamine 5 mg/kg. s.c. twice a day until the morning of the 10th day. One hour after each dose of octylamine 50 mg/kg. of cortisone acetate was injected i.p. On the morning of the 11th day the animals were sacrificed and the ear, lung, small intestine extracted. As it can be seen from Table XI the histamine content of the ear was reduced by 10.9%, that of the lung by 20.8%, and that of the small intestine by 30.2% compared to control values.

In view of the high number of animals which died with the dose of 5 mg/kg. of octylamine, the same sort of treatment was repeated with 9 other animals, but this time only 1 mg/kg. of octylamine was given with cortisone. Tissue histamine contents were reduced as follows: ear, 20.1%; lung, 8.8%; and small intestine, 27.7%.

3) Experiments performed in order to elevate histamine levels of guinea-pigs.

a) Studies with salicylates.

HAINING (1956) has indicated that sodium salicylate reduces in vitro anaphylactic histamine release in rabbit blood. This finding suggested that salicylates might also interfere with

TABLE XI

Treatment: Octylamine 5 mg/kg for 10 days given s.c. twice a day. 1 hour after each dose of octylamine 50 mg/kg cortisone acetate was given i.p.

Animals per Group*	Histamine content of pooled guinea pig organs expressed as the base in µg/gm of fresh tissue		
	Ear	Lung	Small Intestine
2	13.6	16.0	14.3
2	19.5	14.4	13.0
Mean:	16.5 ±2.1	15.2 ±0.6	13.6 ±0.5
Control Mean:	18.4 ±2.4	19.2 ±2.7	19.5 ±1.9
% Difference Control >	10.9%	20.8%	30.2%

* 5 animals died during treatment

normal histamine metabolism, and experiments were therefore undertaken to test their effects on tissue histamine content.

Nine animals were treated orally with acetylsalicylic acid - 30 mg/kg. twice a day until the morning of the 19th day. On the morning of the 20th day the tissues were extracted. The results were as follows: ear histamine level was increased by 8.6%; the lung by 9.4%; and that of the small intestine by 45%. These findings are indicated in Table XII,

Since the water solubility of acetylsalicylic acid is very low, twelve other animals were treated orally with sodium salicylate, 100 mg/kg. given twice a day for 20 days, using a solution containing 15 mg/ml. On the morning of the 22nd day the animals were sacrificed and the histamine of ear, lung, and small intestine extracted. Histamine contents were found to be within the range of the control values.

b) Studies with distilled water.

During the studies with octylamine it was noticed, that animals which were injected with distilled water alone, in order to serve as controls, showed some elevation of their lung histamine levels. It appeared of interest therefore to investigate this point further.

Twelve animals were injected subcutaneously with 0.5 ml. of distilled water twice a day until the morning of the 10th day. On the morning of the 11th day the animals were sacrificed and their tissues extracted.

TABLE XII

Treatment: Acetylsalicylic acid 30 mg/kg orally, twice a day for 19 days

Animals per group	Histamine content of pooled guinea pig organs expressed as the base in µg/gm of fresh tissue		
	Ear	Lung	Small Intestine
3	18.0	26.2	33.3
3	22.0	22.3	26.6
3	--	14.6	25.2
Mean:	20.0 ±1.4	21.0 ±2.7	28.3 ±2.0
Control Mean:	18.4 ±2.4	19.2 ±2.7	19.5 ±1.9
% Difference Control<	8.6%	9.4%	45%

The histamine content of ear, lung and small intestine was examined. As it can be seen from Table XIII the histamine content of the ear and small intestine remained unaltered, while that of the lung was elevated by 52.6% compared to control values.

TABLE XIII

Treatment: Distilled water 0.5 ml (s.c.) for 10 days twice a day

Animals per Group	Histamine content of pooled guinea pig organs expressed as the base in µg/gm of fresh tissue		
	Ear	Lung	Small Intestine
3	20.0	33.7	25.7
3	22.0	34.8	20.8
3	13.9	23.4	16.7
3	16.7	25.2	16.0
Mean:	18.2 ±1.6	29.3 ±2.5	19.8 ±1.9
Control Mean:	18.4 ±2.4	19.2 ±2.7	19.5 ±1.9
% Difference Control	--	52.6%	--

III DISCUSSION

The guinea-pig was chosen as the test animal for the studies designed to achieve either a decrease, or an increase in the histamine content of organs. This choice created a number of rather difficult problems, since it is known, that although the guinea-pig is perhaps the most sensitive of all animals to exogenous histamine, its tissues, in general, neither release histamine very readily, nor contain much histamine. (PATON, 1957). Most of the earlier studies referred to in the literature concerning the physiological role of histamine were indeed performed on rats and it was therefore necessary to establish for each of the procedures to be used the effective range of dosages, frequency of administration, duration of treatment, etc., for the guinea-pig.

In the experiments performed with untreated male and female guinea-pigs of two different strains, it was seen that the average histamine levels of selected organs were higher in female than in male guinea-pigs. This difference was seen in the ear, lung, small intestine, kidney, liver, and heart. Average histamine levels of the spleen appeared to be higher in males than in females. However, that difference seemed to be a feature of the albino strains used.

The most significant difference in histamine contents of female and male guinea-pigs was found in the lungs and small

intestines, where the average levels of female animals were significantly higher than those of male animals. Skin was represented only by histamine determination of the external ear; though higher in the female, the difference was not significant. The same applies to the heart. Both the liver and kidney contain very little histamine in the guinea-pig, so that it is difficult to assess the slight recorded differences.

In studies performed to reduce tissue histamine, it was found that compound 48/80 when given only for a short period of time was unable to cause a reduction in histamine levels of the lung, ear, and small intestine, when tested 24 hours after the last administration of the drug. However, the guinea-pigs showed definite respiratory distress syndrome, approximately one hour after the injection of 48/80. According to MOTA and VUGMAN (1956), 48/80 might have an action on the respiratory mechanism of the guinea-pig, which is not related to histamine release.

The chronic treatment with this compound in increasing doses, indicated some reduction of pulmonary tissue histamine content, which was found to be 18.0% lower than control values. FELDBERG and TALESNIK (1953) have shown that it takes about 45 days before the histamine in the abdominal skin of the rat resumes its normal concentration after depletion by compound 48/80. Their figures show, that restoration is insignificant

for the first 20 days. To test this prolonged action of 48/80 in guinea-pigs, animals were sacrificed on the 7th day following cessation of 48/80 treatment and it was found that the moderate decrease mentioned above was maintained. It was interesting to find, that the histamine content of the ear was reduced by 40.2% seven days after 48/80 treatment has stopped, while the result obtained 24 hours after the last dose showed no reduction in ear tissue histamine levels. This suggests that 48/80 might affect histamine metabolism during the days following the completion of a series of injections - at least in guinea-pig skin. SCHAYER suggested, that cortisone lowers the tissue reserves of histamine in the rat by decreasing its rate of formation. (Histamine, CHURCHILL, London, 1956). When 48/80 and cortisone treatment have been combined in this study, there was a greater reduction in pulmonary and ear skin histamine level than that seen by 48/80 administration alone. While excessively high doses of cortisone were used, it is known (Histamine, CHURCHILL, London, 1956, p.303) that guinea-pigs are in general less sensitive to cortisone than the rat, and rather large doses can be given without toxic effect.

When 48/80 and cortisone treatment was repeated in another group of animals, but extraction of tissues were made only 7 days after the last treatment, it could be observed (Table IX) that reductions in tissue histamine contents were greater than

those achieved with any other treatment.

Octylamine given in doses of 1 mg/kg. for 10 days was ineffective in reducing tissue histamine levels of guinea-pigs. Five mg/kg. given for the same period of time reduced the histamine contents of the ear, lung and small intestine by 28.8%, 34.9% and 18.0% respectively. On the 7th day following cessation of treatment, tissue histamine levels were not different from control values. Combination of octylamine treatment with cortisone administration resulted in less histamine release from the lung and ear than when octylamine was given alone in daily doses of 5 mg/kg., while the histamine content of the small intestine was reduced by 30.2%. HALPERN states (Histamine, CHURCHILL, London, 1956) that in cortisone treated animals release of histamine is hindered. In these studies, cortisone appeared to prevent the full action of octylamine. Since histamine release by octylamine is a slower process than that by 48/80 (PATON, 1957), it might be that cortisone was given too soon after octylamine administration. It seems from the results obtained with 48/80 and octylamine, that these two classis histamine releasers have a different mechanism of action not only in the rat (where 48/80 is believed to act through an enzymatic process while octylamine is thought to penetrate the cell directly and act by ion exchange) but also in the guinea-pig, in which species both 48/80 and octylamine are believed to act as simple organic bases (PATON, 1957).

Salicylates were shown to inhibit anaphylactic histamine release from guinea-pig lung and rabbit blood in vitro (HAINING, 1956). These findings gave the impulse to test their action in experiments which were aimed towards elevating histamine levels in guinea-pig organs. In the small intestine there was a 45% increase of the histamine content obtained by the treatment of acetylsalicylic acid, but the lung and ear values remained unchanged.

Subcutaneous injections of 0.5 ml. of distilled water increased the pulmonary histamine levels of guinea-pigs by 52.6%. Though it is known that hypotonic solutions might have an action on mast cells, this finding cannot be explained.

It is clear from the above findings that the levels of tissue histamine in guinea-pigs can be modified by a wide variety of conditions. The exact underlying mechanisms involved in these modifications are, however, still unclear, and further studies would be necessary before any definite conclusions can be drawn. It is, however, evident that in attempting to assess the influence of pharmacological agents upon tissue histamine, rigidly controlled experimental conditions are essential.

IV SUMMARY

1. Average histamine contents of normal lungs, small intestine, skin of external ear, heart, liver, kidney and spleen were determined in 54 guinea-pigs of both sexes, using two different strains.
2. Average histamine contents of all organs except the spleen were found to be higher in female guinea-pigs. The difference of average histamine content in female lungs and small intestines compared to males was found to be statistically significant.
3. Treatment with compound 48/80 for 3 days proved ineffective in releasing histamine from guinea-pig organs. The administration of this compound to guinea-pigs in increasing doses for 2 weeks, reduced pulmonary histamine levels by 18.3%. This reduction was sustained for at least 7 days, at which time histamine content of the ear was also reduced by 40.2%.
4. By the combined treatment of compound 48/80 and cortisone, greater reduction could be obtained in pulmonary and ear histamine levels, than with 48/80 administration alone for the same period of time. Best results were obtained, when tissue extractions were made 7 days after the cessation of treatment.

5. Octylamine, given in doses of 5 mg/kg for 10 days caused a reduction in histamine levels of the lung, ear and small intestine. These reductions were not sustained for a week. Combined treatment with octylamine and cortisone was less effective in reducing tissue histamine contents than octylamine administered alone.
6. By treatment with acetylsalicylic acid, a 45% increase in the histamine content of the small intestine could be achieved.
7. Subcutaneous injections of 0.5 ml distilled water caused a 52.6% increase in pulmonary histamine levels.

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