SOME OBSERVATIONS ON THE ENZYME

"HISTIDINE DECARBOXYLASE"

IN MAMMALIAN TISSUES.

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INTRODUCTION

In recent years much attention has been given to histamine because of its dramatic pharmacological effects in small doses, and because of its wide distribution in animal tissues. Gaddum (1948, 1951) and Guggenheim (1951) have summarized the knowledge of histamine in their review articles.

It will be found that in spite of much work, there are still many gaps in our knowledge of this substance. It has not yet, for instance, been shown how this histamine is formed in the body.

There appear to be two main theories (Blaschko, 1945). According to the first, histamine is formed in the tissues of the body by the enzymatic decarboxylation of histidine. This histidine is absorbed into the circulation, from histidine taken in with the food, and carried to those tissues in which histamine formation takes place. According to the second theory, histamine is made by bacteria in the alimentary tract, from histidine in the food. The histamine/

histamine is then absorbed into the circulation and carried to the various tissues and stored.

The object of this work is to obtain evidence for the first of these theories, and also to repeat some of the work done on the enzyme by the earlier workers. A brief review of the work, which has already been carried out on this enzymatic formation of histamine from histidine, will be given.

The enzyme concerned in the formation of histamine is called "histidine decarboxylase", which has the power to decarboxylate l-histidine only.

Histidine

Histamine

$$\text{Histidine} \xrightarrow[(-CO_2)]{\text{Decarboxylase}}$$
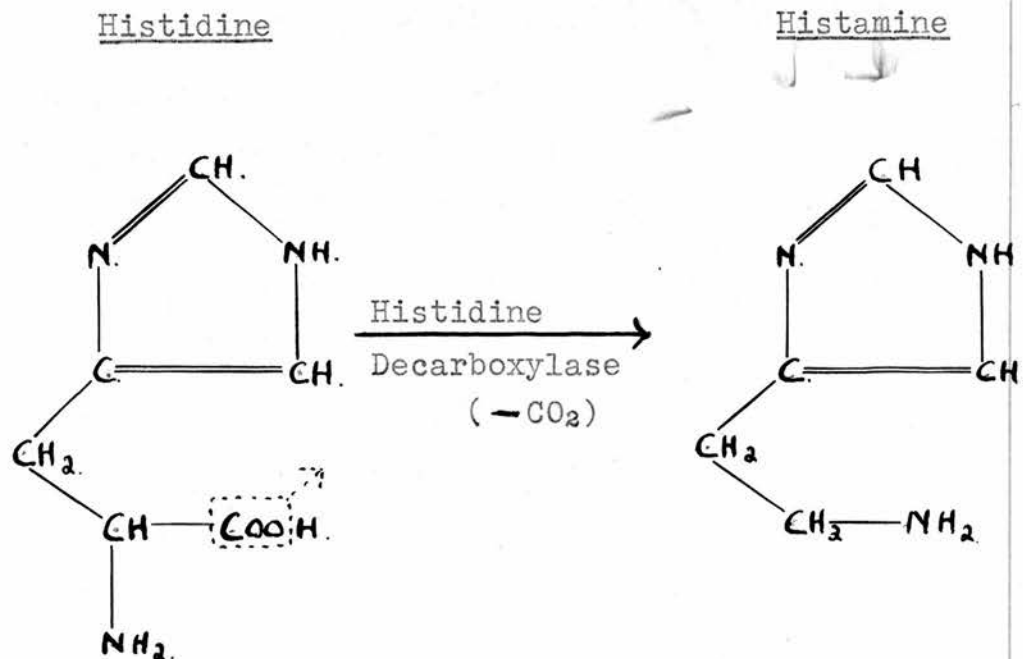
This is not the principle pathway in the break-down of histidine in the body, as this occurs in the liver, due to the enzyme histidase. According to Leuthardt (1951), the imidazole ring is split with the formation of glutamic acid, formic acid and ammonia.

Review of earlier work.

1. On histidine decarboxylase of bacterial origin.

It was during the latter part of the last century that it was realised that some amines were formed during bacterial putrefaction. Müller (1857) isolated isoamylamine from putrefied yeast, which was probably formed by the decarboxylation of leucine. Ellinger (1898, 1900) did the first experiments to induce decarboxylation, by innoculating a synthetic medium with bacteria and incubating for several weeks. He found that putrescine and cadaverine were formed, probably from ornithine and lysine. His experiments were nevertheless inconclusive.

Ackermann (1910, 1911), working on bacterial putrefaction, showed that amines could be formed from certain amino acids by mixed cultures of organisms. He incubated a synthetic medium containing salts,/

salts, peptone, glucose and an amino acid with
decomposing pancreas, and isolated the amine from
the medium after several weeks incubation. Among
other amino acids, he tried histidine and showed the
production of histamine.

Later workers went on to use pure strains of
bacteria. Thus Berthelot and Bertrand (1912) iso-
lated "Bacillus aminophilis intestinalis" which was
capable of forming histamine, tyramine and trypta-
mine from the corresponding amino acids.

Much work has been done on Bacillus coli,
which has been shown to form histamine from histidine.
(Mellanby and Twort, 1912; Kendall and Gebauer, 1930;
Hirai 1933; Matsuda 1933; Gale 1940). The tech-
nique consisted of innoculating a synthetic medium
of salts, histidine and either glycerol or carbo-
hydrate, with a pure strain of Bacillus coli.

Koessler and Hanke (1919) studied the forma-
tion of histamine from histidine by a "colon bacillus"
isolated from a case of cystitis. They showed that
whenever an amine was formed, the medium became
distinctly acid, and they said that histamine was not
formed unless an easily available source of energy,
such as carbohydrate, was available. In (1924) they
further/

further found that the carbohydrate, in being meta-
bolised, was forming the acid medium required, and
so postulated that "amine formation was the result
of an effort by the micro-organism to decrease the
acidity of its environment".

Gale (1940) showed that the production of
amines by bacteria was due to specific amino acid
decarboxylases. He used washed suspensions of
Bacillus coli in a 2% glucose broth. He investigated
many of their properties, including these of an
histidine decarboxylase. Epps (1945) prepared an
acetone dried cell free preparation, having histidine
decarboxylase activity, from Cl. welchii (Type A)
B.W.21. From the results obtained by these workers
most of our knowledge of the bacterial histidine
decarboxylase has been ascertained. As many of its
properties are markedly different from the enzyme of
animal origin those properties have been listed along
with the properties of the histidine decarboxylase
of animal origin.

Epps (1945) tried to resolve the enzyme into
codecarboxylase (pyridoxal phosphate) and apoenzyme,
by methods found suitable for L-lysine decarboxylase
and L-tyrosine decarboxylase, using ammoniacal
ammonium sulphate solutions and dialysis. Normal
dialysis/

dialysis and varying ammonium sulphate ratios were tried, all without success. Epps thus concluded that the histidine decarboxylase appeared to be devoid of codecarboxylase.

Rodwell (1948) could not demonstrate the necessity of codecarboxylase for the decarboxylation of histidine, by Cl. welchii (1948, 1949). Using pure strains of lactobacilli he showed that when these were grown in a pyridoxin deficient medium, there was no reduction in the power of the organism to decarboxylate L-histidine, in fact there was even an increase in activity, but their power to decarboxylate L-lysine, L-ornithine and other amino acids was completely abolished. He thus concluded that codecarboxylase was unlikely to be a component of histidine decarboxylase as had been shown for other amino acid decarboxylases.

This evidence was further supported by Gale (1953) who showed that all the amino acid decarboxylases so far studied, with the exception of histidine decarboxylase, required codecarboxylase as a prosthetic group.

Thus the bacterial histidine decarboxylase appears to be different from the other bacterial decarboxylases/

decarboxylases studied, in at least one important aspect. This variation from other amino acid decarboxylases is also apparent, as will be seen later, with the decarboxylases of animal origin.

2. On histidine decarboxylase of plant origin.

Before discussing the presence of an histidine decarboxylase in animals, it must be mentioned that there is some evidence that one is also present in plants. Werle and Zabel (1947) found histamine in Urticarians, spinach and Chenopodium, but they were unable to prepare an extract containing enzymatic activity. However, they did find that spinach seedlings (especially the roots) when incubated with L-histidine, produced histamine. No histamine was produced when D-histidine was used as the substrate. These results were confirmed by Werle and Raub (1948).

3. On histidine decarboxylase of animal origin.

The first indication of the presence of an animal enzyme capable of decarboxylating histidine with the formation of histamine, came when Bloch and Pinbesch (1936) found an increase in the histamine content of the guinea-pig's lung after an intravenous injection of 200 mg L-histidine. They found that the histamine content of the lungs of control animals varied/

varied from 15-25 µg histamine/gm tissue, while after the intravenous injection of histidine, this rose to 30-45 µg histamine/gm tissue.

This work was repeated by Mackay (1938) who was unable to confirm it. SHe found that the histamine content of the lungs of the control animals was not nearly so constant as Bloch and Pinḃesch found. Mackay's work was supported by that of Holtz and Credner (1944) who could find no increase in the histamine content of the lungs after an intravenous injection of histidine, but they did find a substantial increase, some 10-20-fold, in the urinary excretion of histamine. This excretion of histamine after the parenteral injection of histidine, seemed to prove that the histamine was formed as one of the metabolic products of that amino acid in the animal body.

After the work of Bloch and Pinḃesch, first Werle (1936) and then Holtz and co-workers (Holtz and Heisẞe, 1937b) showed that histamine could be found in the supernatant fluid, when slices of rabbit kidney were incubated with histidine in a buffered Tyrode's solution.

Werle and co-workers (Werle, 1936, 1940, 1942; Werle and Herrmann, 1937; Werle and Krautzun, 1938; Werle and Heitzer, 1938; Werle and Bäumer, 1940; Werle/

Werle and Koch, 1949) have carried out extensive
investigations into the distribution of the enzyme
in the various organs from a variety of animals,
and into many of its physical and chemical properties.
The effects of a large number of substances which
produce a decrease in the activity of the enzyme were
also investigated. An attempt was made by Werle and
Heitzer (1938) to isolate the enzyme. In this, how-
ever, they were unsuccessful, although they claimed
to have effected a purification of about 35-fold.

Holtz and his co-workers (Holtz and Heise,1937b,
1937c; Holtz, Heise and Spreyer, 1938; Holtz,
Credner and Reinhold, 1939; Holtz and Credner, 1941,
1944) also carried out a series of experiments along
the same lines and tested a large number of tissues
from a variety of animals. (This work by Holtz and
his co-workers has been summarized by Holtz (1937:
1941) . A table of the properties of the enzyme of
animal origin has been prepared from the results of
Werle and Holtz. For comparison, are included the
properties of the bacterial enzyme as given by Gale
(1940) and Epps (1945).

4./

4. <u>A summary of the properties of the animal and</u>

<u>bacterial histidine decarboxylases.</u>

a. <u>Contrasting properties</u>

Enzyme of Animal Origin	Enzyme of bacterial origin
1. Unstable	1. Comparatively stable as acetone dried powder.
2. Optimum pH 8.5. Limits pH 6.0 — pH 9.5	2. Optimum pH. Cell free extract 4.5. Cell extracts according to strain 3.0-4.5 Limits pH 2.0 — pH 5.5
3. Optimum molar concentration of substrate 1.6 M.	3. Optimum molar concentration of substrate 0.03 M.
4. Optimum reaction temperature $37^{\circ}C$ - sensitive to temperature change.	4. Optimum reaction temperature $25^{\circ}C$. Not very sensitive to temperature change.
5. Optimum reaction time - 2-3 hours.	5. Optimum reaction time - 1 hour.
6. Histamine formation does not bear a linear relationship to the enzyme concentration.	6. Histamine formation, up to a level, bears a linear relationship to the enzyme concentration.
7. Very small quantities of histidine.decarboxylated (See property 18).	7. Large quantities of histidine decarboxylated.
8. Calculated Q_{CO_2} 0.00861	8. Found Q_{CO_2} 3-60 varying with strain.
9. Acetone ether drying at room temperature causes complete inactivation. Werle (1951) reports some success in acetone ether drying at $-10^{\circ}C$.	9. Acetone ether dried powders, containing the enzyme, are stable and contain full enzyme activity.

b. Common properties

10. Specific to L-histidine.   No other amino acid or histidine homologue decarboxylated.

11. No loss of activity on dialysis.

12. Impossible to resolve the enzyme into codecarboxylase and apoenzyme.

13. Inhibited by heavy metals such as copper, iron, mercury, silver and lead.

14. Inhibited by ketone reagents such as hydroxylamine, hydrazine, semicarbazide, etc.

c. Properties reported only for the
decarboxylase of animal origin.

15. Inhibited by hydrocyanic acid, Suramin (Bayer, 205), thiamine, bile, adrenaline, noradrenaline, D-histidine, L- and D- dopa.

16. Inhibited by pyridoxal-3-phosphate potentiated by pyridoxal-5-phosphate.

17. In electrophoresis, slightly acid.   Enzyme $\longrightarrow$ Cathode.
    Isoelectric point, 6.5 - slightly alkaline - Enzyme $\longrightarrow$ Anode.

18. There is a very small conversion of histidine to histamine, e.g. about 10 μg histamine from 15 mg histidine for 1 gm tissue.   Other animal amino acid decarboxylases produce larger quantities of amine.   E.g. 2 mg tyramine formed from 15 mg L-tyrosine and 10 mg of L-dopa are decarboxylated under similar conditions, when the amino acid was incubated with the appropriate tissue in a Tyrode's medium.

5./

5. Distribution of the animal histidine decarboxylase.

The distribution of the animal histidine decarboxylase in the various tissue of several species of animals, as found by Holtz and Werle has been summarized in a paper by Werle (1943) and is reproduced in Table I.

TABLE 1

The distribution of histidine decarboxylase.

Animal	Tissues in which enzyme found	Tissues in which enzyme not found
Guinea-pig	Kidney ++++ Liver ++ Gut ++++ Pancreas + Stomach + Colon, Caecum, Rectum +	Spleen, heart, brain, skin, lung, adrenals.
Rabbit	Kidney ++++ Liver ++ Gut + Pancrease +	Spleen, heart, brain, skin, lung, adrenals, skeletal muscle.
Rat		Kidney, gut.
Mouse	Kidney ++ Liver +	Gut, lung.
Cat	Kidney + Liver +	Gut.
Dog	Kidney	Kidney, gut.
Hog	Kidney + Liver + Gut $\pm$	
Sheep	Kidney +	Gut.
Horse		Kidney, pancreas.
Cow	Kidney $\pm$	Gut.
Hen	Kidney + Liver +	Lung, pancreas, spleen $\pm$
Hamster	Kidney ++ Liver + Pancreas +	
Pigeon		Kidney, liver.
Man	kidney $\pm$	Liver, lung, caecum, small intestine, mucous membrane of the stomach, gall bladder, skin.

The number of crosses gives the approximate proportion of the enzyme activity.

6. Theories of structure and action of enzyme.

For those enzymes for which it has been demonstrated that codecarboxylase was the prosthetic group, the addition of codecarboxylase to their incubation mixtures potentiated their action. Further, the enzymes were inactivated by dialysis and could be reactivated by the addition of codecarboxylase. However, neither of these properties were found for histidine decarboxylase, in fact, the addition of codecarboxylase to incubation mixtures actually produced an inhibition. Thus it did not appear that codecarboxylase was its prosthetic group. This greatly retarded the work on its structure and action. Even to-day, the structure and action of this enzyme are still uncertain.

Most of the suggestions about the structure of the enzyme and the mechanism of decarboxylation have been put forward by Werle and his co-workers (Werle 1940c, 1942, 1943, 1947; Werle and Heitzer, 1938; Werle and Koch, 1949). Werle and Koch (1949) revised all the previous conclusions and suggested that since pyridoxal-3-phosphate inhibited the enzyme when added to incubation mixtures containing the enzyme, while pyridoxal-5-phosphate potentiated the action, pyridoxal-5-phosphate must be the active group and gave the following structure.
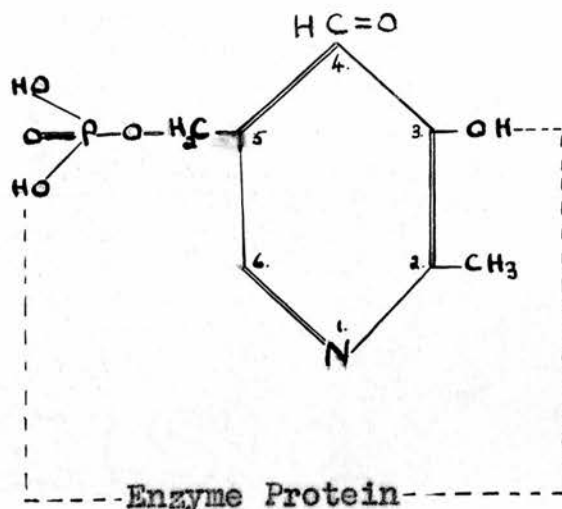
Werle and Koch (1949) also outlined a method by which they believed decarboxylation took place. This involved the formation of a Schiff's base from a molecule of the enzyme and a molecule of histidine. This Schiff's base is decarboxylated. The resulting decarboxylated Schiff's base may then react with a molecule of water to yield the free enzyme and a molecule of histamine. Alternatively, it may react with another molecule of histidine to yield the original Schiff's base and a molecule of histamine.

In support of this theory Werle and Koch (1949) showed that when codecarboxylase, without the apo-enzyme, was allowed to react at room temperature with a neutral aqueous solution of histidine, an intense/

intense yellow solution of the Schiff's base was obtained. The solution contained no detectable histamine. On warming to 60°-70°C. the yellow colour disappeared and a 5-7% yield of histamine was detectable in the solution.

This theory put forward by Werle and Koch (1949) discounted earlier theories (Werle, 1940, 1942, 1943; Werle and Heitzer, 1923). Other theories on the mode of action of histidine decarboxylase have been put forward by Knoops (1938) and Holtz (1937a, 1941). These have been criticised by Werle (1943).

7. Recent work on the histidine decarboxylase of animal origin.

Very little work has been done recently on the enzyme histidine decarboxylase in animals. Martin, Graff, Brundel and Bieler (1949a) tested a series of vitamin P compounds for their effect on the action of histidine decarboxylase in vitro using extracts of guinea-pig kidney as the enzyme preparation. Only a few were active as inhibitors. Quercetin and d-catechin 100%, esculetin and homo-eriodictyol 30% and ascorbic acid 15%, all in 10^{-3} M solution. As these compounds inhibit histidine decarboxylase, which they presume to be responsible for the formation of histamine, a compound known to have/

have an effect on capillary permeability, they
suggest that the vitamin P compounds may play a part
in the maintenance of capillary strength.

The same authors (1949b) tried the effect of
certain metabolic antagonists on histidine decarboxy-
lase.   These had no inhibitory effect on the action
of the enzyme, in fact pyrithiamine and 7-methyl
folic acid showed a potentiation of about 10-15%.
The phenolic compounds tested showed an inhibition
dependant on the number of phenolic hydroxyl groups
present.   Compounds structurally related to
histidine, showed no rigid specificity, compounds
having quite diverse structures being effective
inhibitors.

Werle and Gleissner (1951) showed both in vitro
and in vivo that the histamine contained in the
sting of the bee was formed by an inherent histidine
decarboxylase, which acts specifically on the l-iso-
mer.

Werle and Zeisberger (1952) tested the mucous
membrane of the various parts of the stomach of man,
pig, dog and guinea-pig, and found that there was
definite histidine decarboxylase activity in the
living cells of the upper part of the stomach.

The most important recent work on histidine
decarboxylase has been carried out by Schayer (1952a,
1952b/

1952b) who used radioactive histamine and histidine in several *in vivo* experiments. This important work is discussed later, for it provides evidence in favour of a true histidine decarboxylating enzyme present in mammalian tissues.

8. Evidence that the histamine in animal tissue is wholly or partially derived from histidine by a histidine decarboxylating enzyme.

It must now be considered what evidence there is that the histamine in the body is totally or partially derived from histidine absorbed into the body, and decarboxylated in one or more tissues, into histamine.

It has been suggested that histamine is stored by many tissues, and that the release of histamine, during tissue damage, may have arisen from such stored material, rather than by direct decarboxylation of histidine. The decarboxylation of histidine by mammalian tissues is so small that its significance in histamine release is difficult to assess. Histamine formation by bacteria *in vivo*, on the other hand, is of possible significance. However, the possibility that the production of histamine by decarboxylation of histidine in mammalian/

mammalian tissues is a continuous process and that
the histamine is then stored until its release, due
to injury or other physical process of the tissue
must be considered. Formation and storage may be
in one tissue, or the histamine may be formed in
one tissue and then stored in another tissue.

As has already been stated, some observers doubt
the formation of histamine in the body, maintaining
that it arises as a direct result of bacterial
action in the lumen and is then absorbed into the
body and stored.

There is some evidence to support the theory of
the formation of histamine in mammalian tissues.
Slices of rabbit and guinea-pig kidneys and some
other tissues will produce histamine when incubated
with histidine, even although this figure is very
minute, some 10-15 μg histamine per gram of tissue
for an incubation of 3 hours with 15 mg histidine.
(Werle, 1937; Werle and Krautzun , 1938; Holtz and
Heise, 1937a, 1937b).

Considering the observation that the urinary
excretion of histamine rises after the parenteral
injection of histidine (Holtz and Credner, 1944),
if, as Adam (1950) believes, only 1% of the histamine
formed is excreted in the urine, the increase of
about/

about 20 µg/ml urine 2-3 hours after injection, represents a considerable production of histamine in the body.

The presence of histaminase[*], in large quantities has been shown in the lumen wall (Best and McHenry, 1930). Histaminase is the enzyme which destroys histamine, and any histamine which is absorbed from the gut, for storage in the body, must pass it. It has been suggested that the histaminase is present to prevent the entry of histamine.

Considerable support for the theory of a histidine decarboxylating enzyme present in mammalian tissues, has been provided by the work of Schayer (1952b), who used histamine and histidine labelled with a C^{14} atom in the '2' position of the imidazole ring.

Guinea-pigs were given a single intravenous dose of C^{14} l-histidine, which was considered to be small when compared with the dietary histidine normally taken into the body. C^{14} histamine was found for long periods in those organs rich in bound histamine, such/

[*] The word histaminase has been used through this thesis to indicate any enzyme or enzymes which destroy histamine in the mammalian body. Zeller (Zeller, Fouts and Voegtli, 1953) believes that histaminase and diamine oxidase are the same: Kapeller-Adler (1949) believes that they are different and Schayer (1953a) also speaks of two enzymes.

such as the lungs, liver, intestine and kidney. C^{14}
histamine was also detectable in the urine for
several days following the injection.

Another group of guinea-pigs were injected
intravenously with a dose of C^{14} histamine, calculated
to give a blood level many times greater than in the
previous experiment. Schayer was unable to detect
any C^{14} histamine either in those organs already
mentioned in the previous experiment, or in the
urine.

From these experiments it would appear that free
histamine is rapidly destroyed in the blood and none
is stored. Therefore it seems unlikely that free
histamine is absorbed from the gut. However, if
histidine is absorbed into the body, it can be
converted into histamine and stored in the tissues.

Schayer (1952b) estimated the average amount of
C^{14} histamine reaching the organs daily by sacrifice
of a series of guinea-pigs at varying time intervals
after an injection of C^{14} histidine. He found this
to be 0.04 μg histamine per 1 mg histidine. If he
assumed the normal daily intake of histidine to be
about 80 mg then roughly 3 μg of new histamine were
added to the organs each day. To maintain equi-
librium he assumed that the same amount must be lost
each/

each day.   Assays of the organs such as lung, liver,
intestine and kidneys showed that their total hista-
mine content was about 230 µg histamine, thus the
turnover of histamine is slow, and is in the order
of 50-60 days.   The results of the histamine con-
tent of organs from animals killed 42-56 days after
the injection of C^{14} histidine, Schayer calculated
to be in accordance with his estimated results of the
half-life of histamine.   Thus Schayer estimated
that sufficient histidine was absorbed from an
animal's food to account for all the histamine
formed and present in its body.

One of the chief problems which must be solved
is where the histidine decarboxylase is acting,
whether there is one main organ responsible for
histamine formation, or if there are several organs,
or finally, if all organs which contain histamine
have the power to decarboxylate histidine.

To the present date, there does not appear to
have been any report of an elevated histamine
content in the venous flow from any single organ,
which, considering the quantity of histamine in the
body, and the sensitivity of test preparations to
histamine, one might expect to find if a single
organ was responsible for the formation of histamine.
One/

One must however, consider the possibility that the histamine might be present in a biologically inactive form.

Histamine may be formed in several organs and destroyed in others, or it may be that all organs containing bound histamine have the power to form histamine. The results in this latter case are complicated by the presence of histaminase, which is the chief histamine destroying enzyme. It may be that some organs contain histidine decarboxylase and histaminase, and that the histamine first formed in the cell is not in a form which is attacked by histaminase, but any exogenous histamine would be readily destroyed. There are organs such as the lungs, which have a definite histamine destroying activity, yet having a considerable histamine content. It is possible that the lungs may be purely a histamine-destroying organ, the histamine present having been formed in other organs.

The following studies have been carried out in order to try to solve these latter problems and to obtain more evidence for the existence of this enzyme and to obtain further knowledge of its properties.

PART I

Investigation of the best experimental procedure to show the production of histamine from histidine by mammalian tissue.

Of the mammalian tissues examined by Werle and Herrmann (1937), those with the highest histidine decarboxylase activity in proportion to their weights, were the kidneys of the rabbit and the guinea-pig. Since these tissues were readily available, it was decided to use preparations of rabbit and guinea-pig kidneys as a source of the enzyme for the study of the properties of the mammalian histidine decarboxylase.

The production of histamine on incubation of such preparations with histidine were investigated using the method described by Werle and Herrmann (1937). The preliminary experiments were designed to determine the conditions necessary to give maximal yields of histamine, governing factors, such as the quantity and nature of the tissue preparation in the incubation mixture, the pH of the incubation mixture and the concentration of histidine in the incubation mixture, being examined.

Based on the results of these experiments there was/

was evolved a standardised technique for the
investigation of the histidine decarboxylase activity
of other tissues, and for the examination of the
effect of various substances on the activity of the
enzyme.

a. Measurement of the histamine produced as a
result of enzymatic action on histidine.

This has been carried out in all
experiments by biological assay using the guinea-
pig's isolated ileum suspended in atropinised Tyrode's
solution.

A guinea-pig, weighing from 150 g - 300 g.
was killed by a blow on the head and the lower part
of the ileum, near the ileo-caecal junction dissect-
ed out, perfused with Tyrode's solution to remove
any contents of the ileum, and placed in a petri
dish containing Tyrode's solution.   It was then
freed of all mesentary.   A piece of ileum, about
one inch long, was cut about one inch away from the
ileo-caecal junction.   A piece of cotton was
attached to the wall away from the mesentary at one
end, at the other end a loop of cotton was attached
to the wall away from the mesentary.   The piece of
ileum was suspended in a 2 ml organ bath, by the
loop to a hook at the bottom of the bath, and by
the/

the cotton to a finely balanced lever, writing on a
smoked drum. The bath was filled with Tyrode's
solution containing atropine sulphate (0.1 mg/l).
Air was constantly bubbled through the bath from a
fine jet. The bath was surrounded by water at 37°C.

The mixture was assayed against a standard
solution of histamine acid phosphate containing
0.1 μg/ ml of histamine base. Doses of test and
standard were given alternatively to the bath at
90 seconds interval and allowed to act for 20
seconds before washing with Tyrode's solution. A
response of the standard greater than, equivalent to
and less than a fixed dose of test was obtained.
From this the approximate histamine content of the
test was obtained within definite limits. The
results being expressed in terms of the base.

To determine whether the substance present
in the test fluid causing a contraction of the
ileum, was in fact histamine or some other substance,
0.2 μg mepyramine maleonate was added to the bath
30 seconds before a response of the test, equivalent
to a known dose of standard, was added (Reuse, 1947).
The/

The standard and unknown were added alternatively in
equi-active doses every 90 seconds. The mepyramine
inhibits the response of the ileum to the histamine.
The ileum slowly recovers its sensitivity to hista-
mine. If the test and the standard recover equally,
then the test must contain histamine or a histamine-
like substance.

The incubation mixtures were prepared for bio-
logical testing by adjusting the pH to 5.5-5.9
(short range indicator paper) by the careful addition
of 1N HCl. The mixture was brought to the boil.
This coagulates the proteins. After cooling, the
mixture was filtered through Watman No.1 filter
paper and the filtrate after neutralisation with
solid sodium bicarbonate used for biological assay.

In all calculations of the histamine content
of the mixtures the total volume included the water
volume of the tissues and the volume of the acid
added, usually about 0.45 ml. as well as the total
volume of fluid used in the incubation.

b. Test incubation.

A rabbit was killed by a blow on the
back of the head and then its throat was cut. The
abdomen was opened and the kidneys removed and
placed in 0.9% saline. The kidney capsule was removed
and/

and in so doing, all the adhering fat was also removed.
The kidney was then cut into the thinnest slices possible,
with a razor blade, as described by Umbreit, Burris and
Stauffer (1946). The slices were estimated to be about
1/100th inch thick. Incubation mixtures were then made
up as shown in Table 2 in 25 ml conical flasks.

TABLE 2

Incubation mixtures for the test incubation.

Mixture No.		Wet wt rabbit kidney tissue mg	Tyrode's solution ml	M/5 Na2 HPO4 solution ml	Histidine solution		H2O	
					Before Incubation ml	After Incubation ml	Before Incubation ml	After Incubation ml
1	Tissue test	400	4	2	1	-	-	1
2	Tissue blank	400	4	2	-	1	1	-
3	Reagent "	-	4	2	1	-	-	1

The tissue blank was incubated in order to
determine how much histamine was released from the tissue
in the absence of histidine, that is, the histamine content
of the tissue. The reagent blank was designed to demonstrate
if any histamine originated/

originated from the reagents used. If these two blanks were subtracted from the tissue test, a true result for the histamine, which has been formed from histidine, was obtained.

The M/5, Na_2HPO_4 solution was used to buffer the incubation mixture to pH 8.2 (glass electrode). The histidine solution used was a neutralised solution of l-histidine monohydrochloride in water, containing 15 mg/ml histidine.

The conical flasks were stood in an incubator at 38°C. for 2 hours, and were mechanically agitated. After incubation, the histamine in the supernatant was measured as already described (page 24).

Werle and Herrmann (1937) in their basic incubations, incubated 0.4 g tissue slices in 4 mls Tyrode's solution and 2 mls M/5 Na_2HPO_4 buffer with 15 mg histidine for 2 hours, a total volume of 6.4 ml. In further incubations (Werle and Krautzun, 1938; Werle and Heitzer, 1938) the incubation mixtures were varied considerably.

The above experiment was repeated on a second rabbit, and then again on two separate occasions using guinea-pig kidney tissue. In each experiment, after the histamine content of the incubation mixture had/

had been determined, the test mixtures were tested with mepyramine to determine whether the substance causing a contraction of the ileum was histamine or some other substance.   The results of these experiments are given in Table 3.

The results showed that the individual results for one animal's kidney's slices varied slightly, probably due to variation of tissue thickness.   In spite of this the results showed that there was a wide variation, in the amount of histamine formed, from animal to animal in the same species.   As in the results, rabbit kidney tissue appeared to be more active than guinea-pig tissue, weight for weight, rabbit kidneys were used in most of the following experiments.

The tissue blank was repeated in all the following experiments as the histamine content of the tissue must be known.   In all such cases, the 1 ml histidine solution was replaced by 1 ml of distilled water.   Whenever a new substance was incubated with the tissue mixture, a reagent blank was incubated, this blank will help to determine if the reagent had any effect on the guinea-pig ileum during assay.

c. Tissue form/

TABLE 3

μg histamine found in test incubation mixtures.

TISSUE	Tissue test 1 total histamine μg		Total blank 2 total histamine μg		Reagent blank 3 total histamine μg		Mepyramine test	Histamine μg/g tissue
Kidney slice, Rabbit 1	6.3	7.4	0.03	0.05	nil	nil	+	15.7 - 18.6
Kidney slice, Rabbit 2	2.3	1.9	0.06	nil	nil	nil	+	4.7 - 5.8
Kidney slice, Guinea-pig 1	0.56	0.98	0.06	0.08	nil	nil	+	1.4 - 2.5
Kidney slice, Guinea-pig 2	1.78	1.92	nil	nil	nil	nil	+	4.4 - 4.8

c. Tissue form.

As the use of kidney slices led to variable results in replicate incubations, probably due to variations in tissue thickness, and possibly due to variations in the amount of cortex and medulla of the kidney, which might not have contained the same amount of enzyme, and as minced tissue or a tissue extract appeared to give a more homogenous sample of the tissue, it was decided to try these.

Several slices of rabbit kidney were cut, then the rest of the kidney, excluding the pelvis, was cut up with scissors and minced in a Latapie hand mincing machine. This mincer forces the tissue through small holes against a rotating blade, which cuts the tissue into very fine pieces. The mince was collected in a beaker, stirred to give a uniform mince and aliquots were weighed directly into incubation flasks.

An extract of the tissue was made from the remaining mince by grinding it in a mortar for 5-10 minutes with 1 gm silver sand and 10 mls Tyrode's solution per gram of tissue mince. The fluid was decanted off and centrifuged at 2,500 X g for 10 minutes. The resulting supernatant was called the "rabbit kidney extract" used in these experiments.

Incubation/

Incubation mixtures in duplicate were made up as
shown in Table 4.

TABLE 4

Incubation mixtures for testing the best tissue form.

Test No.	Tissue	Tyrode's solution ml	M/5 Na$_2$ HPO$_4$ solution ml	Histidine solution ml	Water ml
1	0.4 g slice (Slice test)	4	2	1	-
2	0.4 g slice (Slice blank)	4	2	-	1
3	0.4 g mince (Mince test)	4	2	1	-
4	0.4 g mince (Mince blank)	4	2	-	1
5	4 mls extract 0.4 g tissue (extract test)	-	2	1	-
6	4 mls extract 0.4 g tissue (extract blank)	-	2	-	1

The mixtures were incubated at 38°C. for 2
hours then tested for their histamine content. The
whole experiment was repeated using guinea-pig tissue.
The results are given in Table 5.

TABLE 5/

TABLE 5

μg histamine formed by sliced kidneys, minced kidneys and extracts of kidneys from rabbits and guinea-pigs.

TISSUE	μg histamine formed in tissue test.		Mean	μg histamine formed in tissue blank		Mean	Mean μg histamine formed/gm tissue
Rabbit kidney slice	6.73	6.41	6.57	0.31	0.33	0.32	16.4
Rabbit kidney mince	7.02	6.91	6.97	0.33	0.33	0.33	17.4
Rabbit kidney extract	6.51	6.51	6.51	0.33	0.33	0.33	16.3
Guinea-pig kidney slice	2.68	2.84	2.76	0.24	0.24	0.24	6.9
Guinea-pig kidney mince	2.88	2.88	2.88	0.30	0.30	0.30	7.2
Guinea-pig kidney extract	2.32	2.32	2.32	0.28	0.28	0.28	5.8

These results show that there is very little difference in the production of histamine by the different tissue states. The lower results for the extract could be due to loss of some activity in the residue after centrifugation. Slightly more grinding with silver sand remedied this in later experiments. As minced tissue was the most active and easiest to prepare, it was used for most of the following general experiments.

From these results, it would appear that the enzyme readily passed from the tissue into the surrounding medium.

d. Perfused and non-perfused kidney.

Previous experiments had been carried out with tissue containing blood, and it was thought possible that this blood might contain histaminase which might interfere with the maximum yield of histamine (Zeller, 1942). To investigate this point, a comparison was made of the yield of histamine obtained from the kidney tissue of a rabbit, one kidney of which was first freed from blood by perfusion with saline.

The rabbit was killed in the same manner as before,/

before and the abdomen opened immediately.   A
cannula was placed in the left renal artery and
connected to a raised reservoir of 0.9% saline.
The renal vein was cut and the fluid allowed to
escape.   Perfusion was continued until the fluid
emerging from the vein was unpigmented.   The kidney
was then removed. The unperfused right kidney was
removed at the same time.

A tissue mince was prepared from each kidney.
Test and blank incubation mixtures, in duplicate,
were made up as already indicated for minced tissue
in Table 4 and incubated for 2 hours at 38°C.   The
whole experiment was repeated for a second rabbit.
The results of both experiments are given in Table
6.

There was no significant difference between
the histamine productions by the minces prepared
from saline perfused and unperfused kidneys so that
there was no evidence that the blood in the kidney
would influence the results, and therefore it
appeared unnecessary to carry out perfusion of the
kidney, prior to removal, as a routine measure.

From an incubation of whole blood of a rabbit
with histamine, it was found that there was no
histaminase activity, and thus the blood in blood-
rich/

TABLE 6

A comparison of the μg histamine formed by a kidney mince prepared from a kidney perfused with saline and a mince prepared from a kidney containing blood.

TISSUE	μg histamine formed in test mixture.		mean	μg histamine found in tissue blank.		mean	Mean μg histamine formed/gm tissue
Rabbit 1							
Perfused kidney mince	8.9	9.2	9.1	0.2	0.3	0.3	22.0
Non-perfused kidney mince	9.3	9.2	9.3	0.5	0.4	0.5	22.0
Rabbit 2							
Perfused kidney mince	1.46	1.46	1.46	0.1	nil	0.1	3.4
Non-perfused kidney mince	1.54	1.54	1.54	6.1	0.1	0.1	3.6

rich tissues would not affect the results.

e. Cortex and medulla of kidney.

When the pelvis of the kidney was removed, two distinct areas were left, the cortex and the medulla. As the distribution of the enzyme in these two areas was not known, each area was tested separately for its histidine decarboxylase activity.

The fresh kidney of the rabbit, after removal of the capsule and the pelvis, was cut into thin slices. The outer cortex was cut away and minced to give the kidney cortex mince. The area where the cortex and the medulla join, was cut away and rejected. The central area left was minced to give the kidney medulla mince. The two minced tissues were incubated in duplicate with histidine for 2 hours at 38°C. Incubation mixtures were made up as for the minced tissue in Table 4. This experiment was repeated for the kidneys of a second rabbit. The results are given in Table 7.

From the results, it would appear that the enzyme was equally distributed in the medulla and cortex of the rabbit kidney. Thus in further incubations these two areas did not need to be separated, but could be minced together to give a single kidney mince.

TABLE 7/

TABLE 7

μg histamine formed by the medulla and the cortex of rabbit kidneys

TISSUE	μg histamine formed in tissue test.		Mean	μg histamine found in tissue blank		Mean	Mean μg histamine formed/gm tissue
Rabbit 1.							
Kidney cortex mince	0.56	0.56	0.56	0.1	0.1	0.1	1.4
Kidney medulla mince	0.58	0.54	0.56	0.1	0.1	0.1	1.4
Rabbit 2							
Kidney cortex mince	4.7	4.7	4.7	0.2	0.2	0.2	18.8
Kidney medulla mince	5.0	5.0	5.0	0.3	0.3	0.3	20.0

f. Tissue weight.

Various weights of minced rabbit kidney
tissue were incubated in duplicate for 2 hours at
38°C.   Incubation mixtures were made up as for the
minced tissue in Table 4.   The results are given
in Table 8.   In calculating the results, allowance
was made for the various tissue volumes involved.

There appeared to be a fall off in the yield
of histamine per gram of tissue with an increase of
tissue weight, which may, in part, be due to
inefficiency of agitation, when more tissue was
present.   Though the use of small weights of tissue
gave the best production of histamine when expressed
as per gram of tissue, the actual amount of hista-
mine formed in incubation mixtures containing a
small amount of a tissue having low histidine
decarboxylase activity might have been too low for
actual assay.   It was thus decided to continue
using 0.4 g tissue in subsequent experiments.

TABLE 8/

The relation between the weight of tissue used and the amount of histamine formed, when rabbit kidney tissue was incubated with histidine.

TABLE 8

Weight of rabbit kidney mince - gms	µg histamine formed in incubation mixture		Mean	µg histamine found in tissue blank		Mean	Mean µg histamine formed/gm tissue
0.2	2.4	2.4	2.4	0.01	0.01	0.01	12.0
0.3	3.9	3.5	3.7	0.01	0.02	0.01	12.3
0.4	4.8	4.6	4.7	0.01	0.01	0.01	11.8
0.5	5.4	5.4	5.4	0.02	0.02	0.02	10.8
0.7	6.8	6.6	6.7	0.04	0.04	0.04	9.6

g. Optimum histidine concentration.

Minced rabbit kidney tissue was
incubated for 2 hours at 38°C. with various amounts
of histidine.   Incubation mixtures were made up as
for minced tissue in Table 4.   The various amounts
of histidine were dissolved in 1 ml distilled water
and neutralised with solid sodium bicarbonate
(to universal indicator).   Very large quantities
of histidine were dissolved in 1 ml distilled water
and 4 mls Tyrode's solution.   A second experiment
was done to repeat the lower quantities of histidine.
The results are given in Table 9.

The optimum histidine concentration was found
to be obtained when 3.75 mg of histidine were added.
Although this gave the optimum histamine production
per mg histidine, the actual result may have been
too low on some occasions for assay.   It was there-
fore decided to use 15 mg histidine in each incu-
bation, as this was about as good per mg histidine,
but gave much larger results in actual incubation
mixtures.

TABLE 9/

TABLE 9

The relation between the amount of histidine added and the quantity of histamine formed, when rabbit kidney tissue was incubated with histidine.

mg histidine added	Molarity of histidine in incubation mixture	μg histamine in mixture Exp. 1		μg histamine in mixture Exp. 2		Mean μg histamine per mg histidine Exp. 1	Mean μg histamine per mg histidine Exp. 2
3.75	0.0019 M	4.1,	4.1	9.6,	9.2	1.09	2.5
7.5	0.0039 M	5.6	5.0	15.4,	16.0	0.71	2.1
11.25	0.0059 M	7.1,	7.1	20.8,	20.0	0.63	1.9
15.00	0.0078 M	9.1,	9.1	25.8,	25.4	0.61	1.75
24.3	0.0127 M	12.9,	12.7			0.52	
48.6	0.0254 M	16.0,	16.2			0.33	
72.9	0.038 M	13.9,	13.9			0.19	
97.2	0.051 M	7.4,	7.0			0.075	
121.5	0.063 M	7.6,	7.2			0.061	
243.0	0.126 M	7.5,	7.5			0.03	

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Figure I
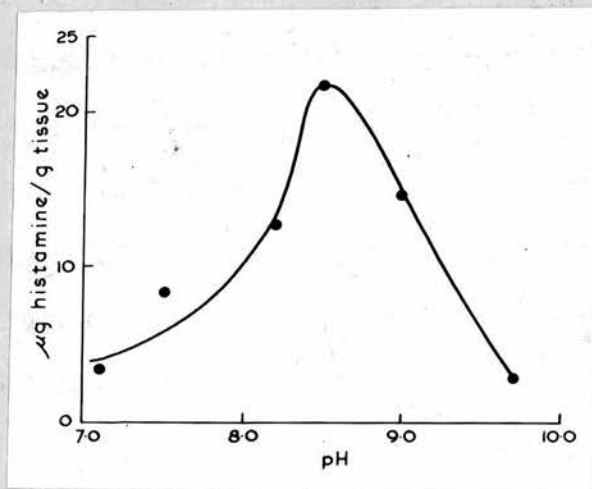
The relation between the histamine
formed and the pH of the incubation
mixture, when rabbit kidney tissue was
incubated with histidine.

h. Optimum pH.

0.4 g rabbit kidney tissue mince was
incubated with 1 ml histidine solution (15 mg/ml)
at various pH's in 4 mls buffered Tyrode's solution
for 2 hours at 38°C.   For pH's greater than 8.2,
2 mls of M/5 Na₂ HPO₄ solution were added.   For
pH's less than 8.2, 2 mls of a mixture of M/5
NaH₂PO₄ (24 g.p.l.) and M/5 Na₂HPO₄ (32.85 g.p.l.)
were added.   1N NaOH solution was added from a
micro pipette to adjust the pH of the incubation
mixture to the required value.   The pH was measured
(glass electrode) before and after the incubation.
Each incubation mixture was made in duplicate.   The
results are given in Table 10.

The results were plotted graphically as shown
in Figure I.   This shows a sharp peak at about
pH 8.5 falling off steeply on either side.

As the production of histamine at pH 8.2 was
good, and as this pH was easier to maintain than
pH 8.5, by use of a phosphate buffer, it was
decided to use pH 8.2 for further incubations.   At
pH 8.5 the peak is very sharp and a small variation
in pH would cause a large variation in the formation
of histamine, while this will not be so great at
pH 8.2.

TABLE 10

The variation in the quantity of histamine formed from histidine when rabbit kidney tissue was incubated at various pH's.

pH at start of incubation	pH at end of incubation	Average pH	µg histamine formed per gm tissue		Mean
7.3	7.0	7.1	3.2	3.6	3.4
7.6	7.4	7.5	8.3	8.3	8.3
8.3	8.1	8.2	12.3	12.9	12.6
8.6	8.3	8.5	22.2	20.8	22.2
9.3	8.8	9.0	14.0	14.7	14.3
10.1	9.5	9.7	2.6	2.6	2.6

i. Optimum incubation time.

The rate of production of histamine
from histidine by minced rabbit kidney tissue was
investigated. 0.4 g minced rabbit kidney tissue was
incubated with 4 mls Tyrode's solution and 2 mls M/5
Na_2HPO_4 solution and 1 ml histidine solution (15 mg/
ml) for varying time intervals, up to 48 hours.   To
each incubation mixture one drop (20 mg) chloroform
was added as a bacteriostatic agent to prevent the
growth of bacteria, which might affect the results
considerably.   The results of this experiment are
given in Table 11.   Each incubation mixture was made
in duplicate.

The whole experiment was repeated using a
kidney tissue extract, prepared as described on page
31.   4 mls of tissue extract was incubated with
2 mls M/5 Na_3HPO_4 solution and 1 ml histidine solu-
tion (15 mg/ml) for short periods of time up to 3
hours.   Each mixture was incubated in duplicate.
(In this experiment, no chloroform was present).
The results of this experiment are given in Table 12.

TABLE 11/

Figure II
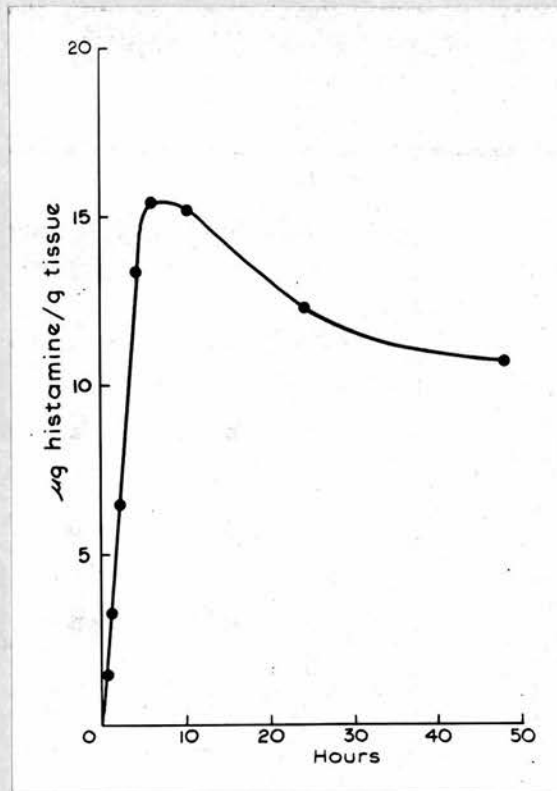
The relation between the histamine formed and the time of incubation when rabbit kidney tissue was incubated with histidine up to 48 hours.

TABLE 11

The relation of the histamine formed to the time of incubation for incubations up to 48 hours, of rabbit kidney tissue and histidine, with chloroform added as an anti-bacterial agent.

Time of incubation (hours)	μg histamine formed per g tissue		Mean
$\frac{1}{2}$	1.5	1.5	1.5
1	3.3	3.3	3.3
2	6.7	6.3	6.5
4	13.3	13.3	13.3
6	15.4	15.4	15.4
10	15.4	15.0	15.2
24	12.7	11.9	12.3
48	10.7	10.7	10.7

TABLE 12/

Figure III
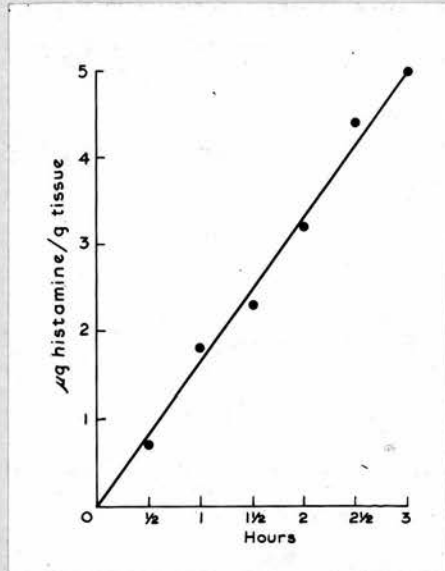
The relation between the histamine formed and the time of incubation, up to 3 hours, when rabbit kidney tissue was incubated with histidine.

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TABLE 12

The relation of the histamine formed to the time of incubation of rabbit kidney with histidine (no chloroform) up to 3 hours.

Time of incubation (hours)	μg histamine formed per g tissue		
			Mean
½	0.7	0.7	0.7
1	1.8	1.8	1.8
1½	2.3	2.3	2.3
2	3.2	3.2	3.2
2½	4.2	4.6	4.4
3	5.0	5.0	5.0

These results were plotted graphically. Figures II and III. The graphs showed that up to 4 hours incubation time, the rate of formation of histamine bore a linear relationship to time. On prolonging the incubation time still further, the rate of production of histamine fell off and finally ceased altogether. It was thus decided to make the incubation times for future experiments 3 hours instead of 2 hours as this was a convenient length of/

of time and yielded about 70% of the maximal histamine production.

Late experiments (page 145) showed that for a 3 hour incubation kidney slices were almost as good as minced tissue, as the histidine decarboxylase probably had sufficient time to diffuse completely into the supernatant out of the tissue, and thus gave results as good as those obtained with a kidney mince.

When this experiment was done at first, the effect of chloroform on the production of histamine was not known, and this effect, fully described in later pages of this work, was not taken into account. When, during further tests the effect of chloroform was shown, this experiment was repeated. The full experiment, with results, is discussed on pages 161-166.

Summary/

Summary

The results of these preliminary experiments
on the production of histamine from histidine by
preparations of guinea-pig's and rabbit's kidney in
a buffered Tyrode's solution may be summarized as
follows.

Minced tissue or tissue extracts were found
to yield more consistent results in replicate
experiments than did tissue slices, weight for
weight.   Preparations of rabbit's kidney proved to
be more effective histamine producers from
preparations of guinea-pig's kidneys in the experiments
where they were compared.   However, it will be
observed from the normal incubations in subsequent
experiments that the histamine forming power of
rabbit's kidneys varies enormously from animal to
animal, from 0.25 μg - 31.2 μg/gm tissue.   This
may also apply to guinea-pig tissue.   However, it
was decided to use rabbit kidney tissue for the
general experiments to follow, as a greater weight
of tissue was available from one animal than in the
case of guinea-pigs.

The presence of blood in the kidneys did not
appear/

appear to affect the results, so that routine perfusion of the kidneys before removal was unnecessary. As the enzyme appeared to be equally distributed between the medulla and the cortex of the kidney, this tissue could be minced into a single mince after removal of the pelvis.

Incubations should be of 0.2 g - 0.4 g of a tissue mince in Tyrode's solution buffered at pH 8.5. The optimum histidine concentration was 0.0020 M, while the optimum time of incubation was 4 hours.

Based on the results of these preliminary experiments, the following procedure was adopted for further investigations, with appropriate modifications wherever necessary. Under these conditions, the amount of histamine formed was always sufficient to ensure an accurate estimate being obtained.

These optimal conditions have not been specified by Werle, but they follow the general line of his work (Werle and Herrmann, 1937; Werle and Krautzun, 1938) and are thus considered approximately equal to his findings.

Experimental/

Experimental procedure for basic incubations for further investigations.

The experimental animal was killed by a blow on the back of the head, and the throat cut. The tissue required for investigation was removed, without perfusion, and placed in 0.9% saline. It was freed from all extraneous tissue, cut into small pieces, and minced in a Latapie hand mincing machine. The resulting tissue being called the "tissue mince".

If an extract was required, the minced tissue was ground with about 1 gm of silver sand and 10 mls of Tyrode's solution for each 1 gm of minced tissue. The mixture was ground for 10 minutes, and then centrifuged for 10 minutes at 2,500×g. The supernatant was known as the "tissue extract".

The incubations were as follows: 0.4 gms of the tissue mince were added to 4 mls Tyrode's solution, or else 4 mls tissue extract were used and the mixture placed in a 25 ml conical flask, together with 2 mls M/5 Na_2HPO_4 solution, as a buffer for pH 8.2, and 1 ml of histidine solution (15 mg/ml). The mixture was incubated at 38°C. for 3 hours, with mechanical agitation.

After incubation, 1N HCl was added from a micro/

micro-burette until the pH fell to 5.5-5.9 (short range indicator paper). The mixtures were brought to the boil, thus coagulating the proteins and preventing further enzyme action. The flasks were then allowed to cool and the mixture filtered through a Watman No.1 filter paper. The filtrate was tested for histamine, as already described (page 24).

On some occasions, the incubations were done on one day and the incubation filtrate stored overnight in the refrigerator, the assays being performed on the following day. It was found that histamine activity was not lost from solutions stored overnight in the refrigerator at $0^{\circ}C$.

In the following experiments, only the mean of the duplicate results is given, as the actual duplicate results were so very nearly alike. At the end of each table of results, the standard error (S.E.) of these results has been included, in order to demonstrate the accuracy of the results. Should the difference of the individual results be large, then the actual results are included, instead of the mean.

In order to demonstrate the method of calculation/

calculation of the standard error (S.E.), the actual calculation of the standard error of the results of all the experiments included in this thesis in which duplicate observations were obtained, is given below.

The ratio of the highest result to the lowest result was calculated for each pair of duplicates. Thus if d = logarithm of the ratio of the highest result to the lowest and N = number of degrees of freedom = number of ratios obtained

The variance of logs = $\lambda = \dfrac{\dfrac{d^2}{2}}{N} = \dfrac{0.061811}{790}$

The standard deviation of the logarithm of the mean of each duplicate is estimated as $\dfrac{\lambda^2}{2} = 0.006255$

The fiducial range for the logarithms (P = 0.05) is $\pm 0.006255 \times 1.96 = \pm 0.01226$.

This corresponds to $\pm 2.8\%$ (antilog 0.01226 = 1.028.

This figure represents the estimated range

of/

of error (P = 0.05) for the estimates of histamine
in this thesis.

PART II

The investigation of substances potentiating the decarboxylation of histidine or inhibiting the action of histaminase.

Three reasons may be suggested for the failure to show the presence of an histidine decarboxylase in those tissues, reported not to contain the enzyme. The first and most obvious reason, may be that the tissue does not contain any enzyme. This is not necessarily true for the enzyme may be present, but under the experimental conditions of the incubation of the tissue with histidine, it is not possible to demonstrate its presence. This may be due to the enzyme being present in such small quantities, that the quantity of histamine formed in an *in vitro* experiment is too small to measure, although the quantity of histamine formed *in vivo* becomes significant when histamine is continually produced.

Alternatively, the enzyme may be present in a tissue which also contains a histamine destroying enzyme, such as histaminase. *In vitro*, this enzyme/

enzyme may destroy histamine faster than it is formed, since both enzymes and any histamine present are to be found in the supernatant, while, _in vivo_, the histamine may be separated, or protected in some other way, from the histaminase.

This set of experiments was designed to find chemical compounds which would either potentiate the action of histidine decarboxylase, so that the presence of small quantities of this enzyme could be demonstrated, or selectively inhibit histaminase so that any histamine, formed by the action of the decarboxylase on histidine, would not be destroyed immediately and could thus be estimated.

If two substances could be found, each having one of these properties, they could be incubated simultaneously with various tissues from several animals, and so a true distribution of the enzyme, histidine decarboxylase could be ascertained.

At first, many experiments were performed to try to increase the yield of histamine formed by the histidine decarboxylase found in the rabbit's kidney. Kidney tissue was incubated with various substances at different concentrations for varying periods of time. The first substances to be tested included two substances which Werle (1942) believed might bring/

bring about an apparent potentiation of the action
of histidine decarboxylase by inhibiting any hist-
aminase present.   These were both diamines,
putrescine (butane-1:4-diamine) and cadaverine
(pentane-1:5-diamine).   Two other diamines were
also tried.   Grewe's diamine (Vit.B, pyrimidine)
and agmatine (1-amino-4-guanidobutane).   These,
along with putrescine and cadaverine were suggested
to be histaminase inhibitors by Arunlakshana, Mongar
and Schild (1954) in a communication to the
Physiological Society (1951).

During this work, in the course of incubations
for periods of 8 hours and longer, a single drop
of chloroform was added to the incubation mixtures,
in order to prevent the growth of bacteria, which
might have interfered with the results.   It was
found that this drop of chloroform itself produced
a marked rise in the production of histamine.
Benzene was found to give an even greater rise in
the yield of histamine.   The results for these
substances are given here but,as a separate investi-
gation was made into this phenomenon, they are
described in more detail under a separate section
of this work.

After several experiments, it became obvious
that/

that there was little or no histaminase in the rabbit
kidney, and that experiments done on this tissue alone,
measured only the power of the substance to potenti-
ate or inhibit histidine decarboxylase.

Thus further tests were introduced.   Each
substance was incubated with rabbit kidney in order
to determine its activity on histidine decarboxylase.
After this, the substance was incubated with hista-
mine and fresh minced cat kidney, a very rich source
of histaminase, in order to determine if the sub-
stance had any effect on the histamine destroying
power of histaminase.   By these two tests it was
hoped to find a substance, which at a given concen-
tration had no inhibitory effect on histidine
decarboxylase, but showed 100% inhibition of histam-
inase.

Werle and co-workers (Werle, 1940, 1942;
Werle and Menniken, 1937; Werle and Krautzun, 1938
and Werle and Keitzer, 1938) had already examined a
very long series of histidine decarboxylase
inhibitors, many of these were ketone reagents.
Most of these inhibited both histidine decarboxylase
and histaminase. Werle (1940) claimed a differen-
tial inhibition of these enzymes with two substances:
Girard's reagent "P" (pyridinium-aceto-hydrazide-
chloride) and Girard's reagent "T" (trimethylammon-
ium-aceto-hydrazidechloride).   It was decided to
re-examine/

re-examine these two substances but none of the
others which he tried, because they were the only
substances tried which showed any usefulness as
selective inhibitors of histaminase.

Schuler (1952) tested several compounds as
histaminase inhibitors, and of those the best were
aminoguanidine and hydrazine sulphate.

A number of substances were shown by
Arunlakshana, Mungar and Schild (1954) to potentiate
the action of histamine on the guinea-pig's isolated
ileum.   They also showed that these substances were
histaminase inhibitors.   From these, three guanidine
derivatives - guanidine, methyl guanidine and di-
methyl guanidine, and glyoxaline were selected for
testing, since they appeared to be the most active.

Finally, some of the better histaminase
inhibiting substances were tested for their activity
against a purified acetone dried powder of hog's
kidney, kindly supplied by Dr. Kapeller Adler, which
contains histaminase activity, as a test of their
inhibitory activity against a purified preparation
of the enzyme.

a. Potentiation of histidine decarboxy-
lase activity.

Minced rabbit kidney tissue was incubated with
and without the test substance in a buffered Tyrode's
medium containing histidine.   The incubation mixtures
were made up in duplicate as given in Table 13.

TABLE 13/

Incubation mixtures to test various substances for a potentiatory or inhibitory effect on the normal histidine decarboxylase activity of minced rabbit kidney.

TABLE 13

No.	Kidney tissue g	Tyrode's solution ml	M/5 Na$_2$HPO$_4$ solution ml	Histidine solution ml	Test before ml	Test after ml	Water before ml	Water after ml
1	0.4	4	2	1	1	-	-	-
2	0.4	4	2	1	-	1	1	-
3	0.4	4	2	1	-	-	1	1

The incubations were maintained at pH 8.2 (glass electrode) by $M/5$ Na_2HPO_4 solution. The histidine solution contained 15 mg/ml histidine. The test was 1 ml of an aqueous 10^{-2}, 10^{-3} or 10^{-4} M solution of one of the following substances: Grewe's diamine (Vit.B_1 pyrimidine) putrescine hydrochloride, cadaverine hydrochloride or agmatine sulphate. The incubations were for 3 hours at $38^{o}C$. After incubation, the histamine content of each mixture was determined as described on page 24.

Incubation mixture 1. measured the histamine formed in the presence of the substance under test, while incubation mixture 2. measured the histamine formed in the absence of the test substance, but as this substance was added immediately after the incubation, any effect it may have had on the gut, would equally affect each result for 1.and 2. Thus the difference between these results measured the effect of the test substance on the formation of histamine. Incubation mixture 3. measured the histamine formed in the absence of the test, and so gave a true result for the formation of histamine, as no test was present in the assay mixture to affect the gut during assay. The difference between 2. and 3. measured the effect of the substance under test, on the gut, either to inhibit or potentiate the effect of histamine. The results of these experiments are given in Table 14.

TABLE 14

The effect of Grewe's diamine, putrescine, cadaverine and agmatine on the histidine decarboxylase activity of minced rabbit kidney tissue.

Substance	Molarity	Incubation Mixture 1. μg histamine/gm.	Incubation Mixture 2. μg histamine/gm.	Inhibition or potentiation of histamine formation.	Incubation Mixture 3 μg histamine/gm.	Effect on gut
Grewe's diamine	10^{-2}	6.1	6.5	nil	6.3	nil
"	10^{-3}	7.9	8.2	nil	8.2	nil
"	10^{-4}	7.8	8.1	nil	8.2	nil
Putrescine	10^{-2}	7.5	7.0	nil	7.3	nil
"	10^{-3}	3.4	3.3	nil	3.3	nil
"	10^{-4}	3.3	3.2	nil	3.3	nil
Cadaverine	10^{-2}	10.4	10.7	nil	9.5	potentiation
"	10^{-3}	9.6	9.4	nil	9.3	nil
"	10^{-4}	9.2	9.3	nil	9.3	nil
Agmatine	10^{-2}	21.8	20.8	nil	13.5	potentiation
"	10^{-3}	20.0	20.0	nil	13.5	"
"	10^{-4}	18.0	18.2	nil	13.5	"

Each result is the mean of duplicate experiments.   S.E. 3.8%

The results showed the histamine yield to be unaffected by the presence of Grewe's diamine, putrescine, cadaverine or agmatine in the incubating mixture in concentrations of 10^{-2} M. Even incubations up to 45 hours with 10^{-2} M Grewe's diamine, failed to demonstrate any increases in yield.

In view of the long incubation times used in this last experiment, one drop (20mg) chloroform was added to the incubation mixture as a bacteriostatic agent. A subsequent incubation showed that chloroform was not acting only as a bacteriostatic agent, but that it had a direct effect on the production of histamine from histidine. A similar, but even greater effect was noted when benzene was used instead of the chloroform.

Incubation mixtures were made up in duplicate as given in Table 15. Each mixture was incubated for 3 hours at 38°C.

The histidine solution contained 15mg/ml histidine. One drop of chloroform or benzene weighed approximately 20 mg.

TABLE 15/

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TABLE 15

Incubation mixtures to show the effect of benzene or chloroform on histidine decarboxylase activity.

No.	Kidney tissue mince gm	Tyrode's solution ml	M/5 Na₂HPO₄ solution ml	histidine solution ml	water ml	chloroform or benzene before	chloroform or benzene after
1	0.4	4	2	1	1	1 drop	-
2	0.4	4	2	1	1	-	1 drop
3	0.4	4	2	-	2	1 drop	-

Incubation mixture 1. measured the formation
of histamine in the presence of either chloroform
or benzene.   Chloroform and benzene were added
after the incubation, as in mixture 2, in order to
make any effect, due to either of these substances,
which might have been left after bringing the
mixture to the boil, the same for each mixture.
Mixture 3. served as a control of the histamine
release or formation in the absence of any histi-
dine.

The results of these experiments are given
in Table 16.

The results demonstrated that chloroform,
and to an even greater extent benzene, caused a
significant increase in the formation of histamine
from histidine, which could not be accounted for
by a release of histamine from the tissue, or an
effect on the gut, as the results of incubation
mixture 3. and 2. respectively, showed.

TABLE 16/

TABLE 16

The potentiating effect of chloroform and benzene on the production of histamine from histidine by minced rabbit kidney tissue.

Incubation mixture 1. Test before µg histamine/gm.	Incubation mixture 2. Test after µg histamine/gm.	Ratio 1/2	Incubation mixture 3. No histidine µg histamine/gm.	Ratio benzene/ chloroform
Tests with chloroform alone:				
19.7	6.7	2.98	0.8	
34.4	10.8	3.19	0.5	
6.8	1.6	4.2	0.1	
11.1	3.2	3.5	0.2	
Chloroform and benzene tested on aliquots of the same tissue:				
(Chloroform: 0.97	0.25	4	0.01)	
(Benzene: 10.0	0.25	40	0.01)	10
(Chloroform: 24.9[+]	7.6	3.5	0.4)	
(Benzene: 56.9[+]	7.6	7.9	0.5)	2.3

Each result is the mean of duplicate incubations.

S.E. 2.6%

[+]A mepyramine test (page 25) on each of these mixtures showed that the gut active substance was histamine or a histamine-like substance.

The phenomenon of the potentiation of histamine formation as a result of the presence of chloroform and benzene in the incubation mixtures has been investigated at length and the results form the subject matter of a later section (page 83).

However, the results of these preliminary experiments were sufficient to indicate that the addition of benzene to the incubation mixture would be helpful for the demonstration of histidine decarboxylase activity in those tissues in which the enzyme concentration was low.

A number of substances were studied, not only to determine if they potentiated histidine decarboxylase activity, but also to observe their effect on this enzyme with a view to testing the same substances for their activity as histaminase inhibitors.   The experiments were designed to show up any substances, which at a given concentration, would inhibit by 100% the activity of histaminase, but would be without any inhibitory effect on the histidine decarboxylase activity.

Each of the substances listed in Table 18 was incubated for 3 hours at $38^{\circ}C.$ at 10^{-3} and 10^{-4} M concentrations, with a rabbit kidney mince and histidine, and the histamine yield compared with that/

that obtained in the absence of the test substance.
(The incubation mixtures differed slightly from the
normal incubations in that the total volume was
increased to 10 mls, in order to make it easier to
obtain the final molar concentrations required.)
Incubation mixtures were made in duplicate, as in
Table 17.

The test solution was 1 ml of a 10^{-2} or 10^{-3} M
solution of one of each of the substances listed in
Table 18.   The histamine content of the mixtures
after incubation was determined as described on
page 24.   The results are given in Table 18.

TABLE 17/

TABLE 17.

Incubation mixtures to test various substances for their effect on the normal histidine decarboxylase activity of minced rabbit kidney.

No.	Minced rabbit kidney tissue gm	Tyrode's solution ml	Buffer solution pH 8.2 ml	Histidine solution 15mg/ml ml	Test solution		Water	
					before incubation ml	after incubation ml	before incubation ml	after incubation ml
1. Test	0.4	5.6	2	1	1	-	-	1
2. Test blank	0.4	5.6	2	1	-	1	1	-

TABLE 18

Inhibition of the histidine decarboxylase of minced rabbit kidney. Each result is the mean of duplicate incubations. S.E. 3.7%

Substance	10⁻³ M solution			10⁻⁴ M solution		
	μg histamine formed/gm Test before 1.	μg histamine formed/gm Test after 2.	% Inhibition	μg histamine formed/gm Test before 1.	μg histamine formed/gm Test after 2.	% Inhibition
Glyoxaline	5.9	6.1	0	6.1	6.0	0
Methyl guanidine sulphate	5.9	5.9	0	5.9	5.9	0
Asym. dimethyl guanidine sulph.	6.7	6.4	0	6.1	6.2	0
Amino-guanidine bicarbonate	3.7	6.1	40	6.4	6.4	0
Guanidine carb.	6.7	6.4	0	6.7	6.4	0
Hydrazine sulph.	nil	6.1	100	nil	6.0	100
Girard's reagent P †	1.7	6.0	72	3.9	5.9	32
Girard's reagent T *	2.1	5.9	66	4.8	6.0	21
Marsilid †	1.2	8.8	86	7.0	8.5	17

† Pyridinium-aceto-hydrazide chloride.
* Trimethyl-ammonium-aceto-hydrazide chloride.
† 1-isonicotinoyl-2-isopropylhydrazine.

These results are summarized and described along with the results obtained for the effect of these substances on histaminase activity.

b. Inhibition of the histaminase activity of an extract of cat's kidney.

The substances already mentioned in Table 18 together with the four diamines, mentioned on page 60 were each incubated at different concentrations with cat kidney extracts and histamine and the inhibition of the destruction of histamine noted.

Cat kidney is a rich source of histaminase activity.   Extracts were made by grinding 1 gm of silver sand and 10 mls of Tyrode's solution with every gram of minced cat kidney in a mortar.   The mixture was diluted to 50 mls with Tyrode's solution and centrifuged at 2,500 X g, the super-natant ("cat kidney extract") which contained the equivalent of 20 mg fresh kidney/ml served as the enzyme preparation.

The optimum pH for histamine activity was 7.2. This was obtained by using a M/3 phosphate buffer (24 g.p.l. sodium dihydrogen phosphate dihydrate + 32.85 g.p.l. disodium hydrogen phosphate dihydrate).

Each incubation mixture was incubated for 1 hour/

hour at 38°C. After incubation, the histamine content of the mixtures was determined as for the histidine carboxylase incubations, page 24. Incubation mixtures were prepared in duplicate as indicated in Table 19.

TABLE 19

Incubation mixtures to test the substances
listed in Table 20 for their effect on the activity of histaminase
contained in cat kidney extracts.

	Cat kidney extract	Tyrode's soln.	Buffer pH 7.2	Hista-amine soln 20μg/ml	Test sub.		Water	
					before incubation	after	before incubation	after
	ml	ml	ml	ml	ml	ml	ml	ml
Test (T)	4	1	3	1	1	-	-	1
Control 1 (C_1)	4	1	3	1	-	1	1	-
Control 2 (C_2)	4 (boiled)	1	3	1	1	-	-	1

1 ml of the test substance in the test incubation, was 1 ml of a solution of one of the substances given in Table 20 at one of the following concentrations 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M. In the two control incubations 1 ml of test was 1 ml 10^{-2} M solution/

solution of that particular substance.

The normal destruction of histamine by the histaminase of cat kidney tissue was obtained from the assay of control 1. after incubation. The amount of histamine found in the incubation mixture in the absence of enzyme action was obtained from the assay of control 2, as the cat kidney extract was brought to the boil and then cooled in order to destroy the histaminase activity before being incorporated in the incubation mixture. Thus the difference between the histamine contents of the two control mixtures gave the amount of histamine destroyed by histaminase. The difference between the histamine contents of the 'test' mixture and control 1. gave the amount of histamine which escaped destruction as a result of the presence of the test substance in the incubation mixture. From these figures the percentage decrease of the effect of the histaminase for each concentration of the test substance, was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{T - C_1}{C_2 - C_1} \times 100.$$

The results are given in Table 20.

TABLE 20/

The total histamine (µg) left in incubation mixtures, after incubation of histamine with cat kidney extracts in the presence of several test substances, and the percentage inhibition of the destruction of histamine (calculated from the formula:

$$\% \text{ inhibition} = \frac{T - C_1}{C_2 - C_1} \times 100,$$

produced by the addition of these substances.

TABLE 20

SUBSTANCE	Control 2. µg histamine	Control 1 µg histamine	10^{-7} M Test	10^{-7} M % inhibition	10^{-6} M Test	10^{-6} M % inhibition	10^{-5} M Test	10^{-5} M % inhibition	10^{-4} M Test	10^{-4} M % inhibition
Marsilid	20.7	2.5	2.4	0	2.4	0	2.4	0	3.9	8.2
Glyoxaline	20.0	1.0	0.7	0	—	—	—	—	—	—
Methyl guanidine sulph.	20.7 / 19.3	2.5 / 9.4	—	—	7.8	29.2	14.7	53	19.3	100
Asym. dimethyl guanidine sulph.	20.7 / 19.3	2.5 / 9.4	1.0	0	7.3	26.4	13.6	42	16.8	75
Amino guanidine bicarb.	20.0	1.0	8.1	32	10.5	50	20.0	100	20.0	100
Guanidine carbonate	20.0 / 19.3	1.0 / 9.4	1.0	0	1.3	2	—	9	12.6	32
Hydrazine sulphate	20.7	2.5	15.1	69	19.6	94	20.4	99	20.8	100
Girard's Reagent 'P'	20.7	2.5	2.4	0	4.9	13	11.5	50.2	15.8	73.4
do. 'T'	15.0	1.4	1.4	0	2.6	8.0	4.7	22	10.3	59.2
Grewe's di-amine	20.0	1.6	4.1	13.5	5.4	20.6	16.0	50	16.0	76
Putrescine	20.0	1.6	1.6	0	1.8	0	2.6	5.5	4.5	15.7
Cadaverine	20.0	1.6	1.6	0	1.6	0	1.6	0	5.3	20
Agmatine	20.0	1.6	1.5	0	1.8	0	5.2	14.9	9.1	40.7

The results for these tests for the inhibition of histaminase activity of the cat's kidney (Table 20) and the results for the tests of the same substances on the histidine decarboxylase activity of the rabbit's kidney (Table 18), have been summarized in a single table (Table 21).

TABLE 21

A summary of the results of the inhibitory action of various substances on the histidine decarboxylase activity of the rabbit's kidney and on the histaminase activity of the cat's kidney.

Compound	Molar concen-tration	% Inhibition of histamine des-stroying act-ivity.	% Inhibition of histidine decarboxylase activity
Hydrazine sulphate	10^{-3}	100	100
	10^{-4}	100	100
	10^{-5}	100	
	10^{-6}	94.0	
	10^{-7}	69.0	
Girard's Reagent 'P'	10^{-3}	100	75
	10^{-4}	73.4	37
	10^{-5}	50.2	
	10^{-6}	13.0	
	10^{-7}	0	
Girard's Reagent 'T'	10^{-3}	83.5	66
	10^{-4}	59.2	21
	10^{-5}	22.0	
	10^{-6}	8.0	
	10^{-7}	0	

Grewe's/

Table 21 Contd:

Compound	Molar concen-tration	% Inhibition of histamine des-stroying activity	%Inhibition of histidine decarboxylase activity
Grewe's diamine	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	96 76 50 20.6 13.5	0 0 0
Putrescine	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	51 15.7 5.5 0 0	0 0
Cadaverine	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	31.4 20 0 0 0	0
Agmatine	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	76 40.7 14.9 0 0	0 0
Glyoxaline	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	87 100 53 29.2 0	0 0
Guanidine carbonate	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	76 33 9 2 0	0 0
Methyl guanidine sulphate	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	100 75 42 18.1 0	0 0

Asym/

Table 21 Contd:

Compound	Molar concentration	% Inhibition of histamine destroying activity	% Inhibition of histidine decarboxylase activity
Asym. di methyl guanidine sulph.	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	100 86 53 26.4 0	0 0
Amino guanidine bicarbonate	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	100 100 100 50 32	40 0
Marsilid	10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}	49.2 8.2 0 0 0	86 17

From the results shown in Table 21, it will be observed that 10^{-3} M solutions of methyl guanidine sulphate, and asym. dimethyl guanidine bicarbonate, and 10^{-4} M and 10^{-5} M aminoguanidine bicarbonate, showed 100% inhibition of the histaminase activity in cat's kidney extracts, but showed no inhibition of the histidine decarboxylase activity of rabbit's kidney.

C./

c. Inhibition of the histaminase
activity of a purified dry powder of hog's kidney.

Following the use of histamine, labelled with
a C^{14} atom in the 2-position of the imidazol ring,
Schayer (1953b) showed that there appeared to be
two histamine metabolising enzymes - histaminase
and "histamine metabolising enzyme II". He con-
sidered that in the cat the chief histamine
destroying enzyme was the "histamine metabolising
enzyme II", in rats the chief enzyme was histaminase,
while in guinea-pigs and mice, he believed the
ratio to be about equal.

Kapeller-Adler (1949) prepared an acetone
dried sample of hog's kidney, which was believed
to be a rich source of histaminase.

Because of the uncertainty over the actual
histamine destroying enzyme, and because Schayer's
results (1953a, 1953b) showed that at least one of
the substances, tested in experiments already
mentioned (page 73) inhibited one of these enzymes,
while another substance, also tested, inhibited the
other enzyme (See discussion, page 73). it was
decided to test the inhibitors, already found to be
the most powerful against the histamine destroying
activity of cat's kidney (page 73) against the
histaminase activity of a purified, acetone-dried
powder/

powder of hog's kidney, kindly supplied by Dr. R.W. Kapeller-Adler.

The incubations were carried out at pH 7.2, using the same phosphate buffer as was used in the previous experiments (page 70). The histaminase preparations were prepared by shaking 30 mg purified dry powder of hog's kidney with 20 mls of the phosphate buffer pH 7.2.

Incubation mixtures were prepared in duplicate as shown in Table 22. Each mixture was incubated for 1 hour at 38°C. After incubation, the mixture was brought to the boil to destroy the histaminase. The mixtures were then assayed directly for histamine on the guinea-pig ileum, as described on page 24.

TABLE 22/

TABLE 22

Incubation mixtures to test various substances for their effect on the activity of histaminase contained in the hog kidney preparation.

Number	Histaminase preparation ml before incubation	ml after Incubation	Tyrode's soln. ml	Phosphate buffer pH 7.2 ml	Histamine solution (10g) ml before	ml after	Test substance
1	1	-	4	2	1	-	1
2	1	-	4	2	1	-	-
3	-	1	4	2	1	-	1

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Mixture 1. gives the histamine left in the presence of the test and mixture 2. gives the histamine left in the absence of the test. Mixture 3. is the histamine blank, that is, the amount of histamine in each mixture, in the absence of the histaminase preparation.

The test substance was 1 ml of a 10^{-1} or 10^{-2} M solution of putrescine dihydrochloride, cadaverine dihydrochloride or agmatine sulphate, or a 10^{-3} M solution of aminoguanidine bicarbonate, guanidine carbonate, asym dimethyl guanidine sulphate or Marsilid (page 69) or 1 ml of an aqueous solution of histidine dihydrochloride (15 mg/ml histidine) or finally 1 ml of Tyrode's solution containing one drop of benzene. The results are given in Table 23.

TABLE 23/

TABLE 23

The inhibition of the histaminase activity of a purified, acetone dried preparation of hog's kidney suspended in Tyrode's solution.

TEST SUBSTANCE	Final molar concentration	µg histamine left with test 1	µg histamine left without test 2	µg histamine left histamine blank 3	µg histamine destroyed with test (3-1)	µg histamine destroyed without test (3-2)	% Inhibition of histaminase activity $\frac{(3-2)-(3-1)}{(3-2)} \times 100$
Putresince dihydrochloride	10^{-2} 10^{-3}	10.3 7.8	5.4 5.1	10.1 9.9	nil 2.1	4.7 4.8	100 56 (51)
Cadaverine dihydrochloride	10^{-2} 10^{-3}	9.9 7.2	5.4 5.1	10.1 9.9	nil 2.7	4.7 4.8	100 44 (314)
Amino guanidine+ bicarbonate	10^{-4}	19.1	10.2	19.3	0.2	9.1	98 (100)
Agmatine sulphate	10^{-2} 10^{-3}	10.5 8.9	5.4 5.1	10.1 9.9	nil 1.0	4.7 4.8	100 80 (76)
Guanidine + carbonate	10^{-4}	14.9	10.2	19.3	4.4	9.1	52 (32)
asym. dimethyl+ guanidine sulphate	10^{-4}	16.1	10.2	19.3	3.2	9.1	65 (86)
Marsilid +	10^{-3}	14.4	10.2	19.3	4.9	9.1	46 (49.2)
Histidine mono-hydrochloride		4.4	4.4	10.2	4.6	4.8	0
Benzene + "		4.9 9.1	4.6 10.2	10.2 19.3	4.8 10.2	4.5 9.1	0 0

+ 20µg histamine was added. Each result is the mean of duplicate incubations.
The figures in brackets are the corresponding results for the test against the histamine destroying activity of the cat's kidney (page 73), S.E. 3.2%

It will be seen from these results that the
inhibitory activity of the substances given in
Table 23 against the action of the histaminase of
purified, acetone dried, hog's kidney preparation,
were very similar, and in the same order as the
results obtained when the same substances were tested
against the histamine destroying activity of cat's
kidney.

Thus it was decided to use 10^{-4} M amino guanidine
bicarbonate as a selective inhibitor of histaminase
for further experiments with various tissues from
several animals, in order to study the distribution
of the enzyme histidine decarboxylase, as this sub-
stance was the strongest selective inhibitor of
histaminase activity. Its presence in the incubation
mixture of the tissue in a buffered Tyrode's medium,
to which histidine had been added, would effectively
prevent the destruction, by histaminase, of any
histamine formed from the histidine.

To this incubation mixture, one drop of benzene
could be added, as it had been shown (page 65) that
this organic solvent enhanced the production of
histamine from histidine, and so would help to make
the activity of small amounts of histidine decarboxy-
lase more apparent.

PART III

Experiments on histamine-histaminase rich tissues and the distribution of histidine decarboxylase in several tissues of selected animals.

One of the weakest parts of the theory, which believes that the histamine, found in the body, as derived from histidine by decarboxylation carried out by an enzyme histidine decarboxylase, is the fact that the enzyme has been shown to be present in so few mammalian tissues. There appears to be no single organ with a high histidine decarboxylase activity, irrespective of the species from which it is obtained. Thus the fresh kidneys of the rabbit and the guinea-pig readily form histamine from histidine _in vitro_ and do not readily destroy histamine. On the other hand, the kidneys of the cat and the hog, cow and horse (Werle, 1943) have a powerful histamine destroying activity, and do not appear to possess any histamine forming activity.

The fact that the presence of histidine decarboxylase in any particular tissue has not been demonstrated by simple incubation of the tissue with histidine/

histidine, does not prove that the enzyme is absent.
The activity may for instance, be very weak or its
presence may be masked by an excess of histaminase,
such that in vitro any histamine formed is immedi-
ately destroyed, and so cannot be measured.   This
part of the investigation was designed to test these
two possibilities.

In Part 2 of this research, the object had been
to discover first of all a histidine decarboxylase
potentiating substance, and secondly a selective
inhibitor for histaminase which would not inhibit
the histidine decarboxylase.   Many chemical com-
pounds were tried, and though a histidine decarboxy-
lase potentiator - benzene, was soon found, it was
some considerable time before the selective histam-
inase inhibitor - amino-guanidine was discovered.

In the meantime, many experiments were carried
out on histamine-histaminase rich tissue, in order to
try to show the presence of some histidine decarboxy-
lase.   First of all, lung tissue was incubated with
and without histidine and at the same time with and
without benzene, in order to determine if either of
these substances influenced the destruction of
histamine by the histaminase present.   This was done
because it was found that some tissues,i.e.,rabbit
lung/

lung and ileum, possessed a definite histamine
destroying activity when tested and in vitro,
yet on analysis, extracts of both of these tissues
were found to contain histamine. This histamine
could have been formed in the tissue or brought to
the tissue, for destruction, in the blood stream.

In order to determine if the tissue was
actually capable of forming histamine, tests were
designed to release all the histamine in the tissue
and then to incubate with histidine. If any
histamine was found in the tissue after incubation
with histidine, it could have only been formed from
the histidine. Lung tissue was chosen for these
tests.

Several methods were used to reduce the
histamine content of the tissue to zero. First,
the minced tissue was incubated at 38°C in Tyrode's
solution in order that the histaminase present in
the tissue might destroy the histamine it also con-
tained. The tissue was then incubated with histi-
dine. Alternatively, the histamine was released
from the tissue by electrodialysis and then the
tissue incubated with histidine.

Finally, the histamine was liberated by the use
of a histamine liberator such as octylamine or
decylamine. When the histamine liberator was
washed/

washed out of the tissue, the tissue was incubated
with histidine.   This failed, because any of the
enzyme which was present passed into the histamine
liberator solution, and in this it was inhibited.
The inhibition of histidine decarboxylase activity
by these tissue histamine liberators was demon-
strated using rabbit kidney tissue as a source of
the enzyme.

When it was discovered that amino-guanidine
in 10^{-4} M solution inhibited histaminase but not
histidine decarboxylase, a long series of experiments
were carried out in which many tissues from animals
of several species were incubated in a buffered
Tyrode's solution with one drop of benzene and 10^{-4} M
amino-guanidine, in order to determine if any of
them showed an increase in histamine concentration,
over the original histamine contained in the tissue.
At the same time, the distribution of histamine and
histaminase throughout the tissues was also investi-
gated.

a. Incubation of histamine rich tissues
with histidine and benzene.

These experiments were carried out on rabbit
lung tissue, as it had been noted in preliminary
experiments that this tissue contained a quantity
of/

of histamine, and also possessed a histamine destroying activity.

After the animal was killed in the normal manner (page 50). The thorax was opened and the lungs removed, into 0.9% saline. They were washed in saline to remove all excess blood, then freed of any extraneous tissue including as much of the bronchi as possible, minced in a hand-mincing machine and weighed directly as wet tissue. Experimental incubation mixtures were made up in duplicate as shown in Table 24. After incubation, each mixture was assayed for histamine, as described on page 24. The results are included in Table 24.

In a repeat experiment, incubation times of 3 and 6 hours were used and additional mixtures containing benzene 20 mg were included. The results are given in Table 25.

TABLE 24/

TABLE 24

Incubation mixtures and the results obtained when histamine rich tissue, rabbit lung, was incubated with and without histidine, and the histamine content of the mixtures determined after fixed periods of time.

Test No.	Tissue weight gms	Tyrode's soln. ml	M/3 phosphate buffer pH 7.2 ml	Histidine soln. 15mg/ml ml	Distilled water ml	Incubation time at 38°C. hours	µg histamine formed in incubation
1	0.5	4	2	1	-	No Incubation	9.5
2	0.5	4	2	-	1	"	9.5
3	0.5	4	2	1	-	2	6.3
4	0.5	4	2	-	1	2	2.8
5	0.5	4	2	1	-	3	5.2
6	0.5	4	2	-	1	3	1.9
7	0.5	4	2	1	-	4	3.8
8	0.5	4	2	-	1	4	1.6

Each result is the mean of duplicate incubations.   S.E. 3.4%

TABLE 25

Histamine found in mixtures after incubation of rabbit lung tissue in buffered Tyrode's solution, with and without histidine, with and without benzene.

	μg histamine/gm tissue			
	No histidine added		15mg histidine added	
	Benzene added to the incubation mixture.			
	before	after	before	after
		incubation		
No incubation. Tissue blank	16.8	19.9	17.3	19.9
3 hrs incubation.	6.7	9.4	9.8	18.9
6 hrs incubation.	0.11	7.4	3.6	11.9

Each result is the mean of duplicate incubations.   S.E. 4.2%.

In a further repeat experiment, incubation times of 3 and 5 hours were used.   Incubation mixtures were made up as at table but including a third incubation mixture, to which 150 mg histidine were added during incubation.   The results are given in Table 26.

TABLE 26

Histamine found in mixtures after incubation of rabbit lung tissue in a buffered Tyrode's solution in absence of, or in the presence of 15 mg or 150 mg histidine.

	μg histamine found		
	No incubation	3 hr incubation	5 hr incubation.
No histidine during incubation	48.2	24.5	16.5
15 mg " "	48.2	32.2	20.8
150 mg " "	47.0	32.2	37.6

Each result is the mean of duplicate incubations.   S.E. 5.5%.

A side experiment was done in which 150 mg histidine was dissolved in 7 mls of water, then diluted to the same degree as these samples were diluted for assay and tested on the guinea-pig's isolated ileum.   This showed that the large amount of histidine had no direct effect on the ileum.

In a final experiment, the last experiment was/

was repeated for incubation times of 2, 4 and 6
hours. An additional mixture was included which
contained no histidine but one drop (20 mg) benzene.
The results of this experiment are given in Table 27.

TABLE 27

Histamine found in mixtures after incubation
of rabbit lung tissue, in a buffered Tyrode's
solution, in the absence, or in the presence of 15mg
or 150 mg histidine, and in the absence of histidine
but in the presence of benzene.

	μg histamine found			
	No incubation	2 hr incubation	4 hr incubation	6 hr incubation
No histidine in incubation	15.0	10.4	7.3	6.5
No histidine in incubation but + 20 mg benzene	15.0	10.9	7.7	7.0
15 mg histidine in incubation	15.0	11.6	8.7	7.8
150 mg histidine in incubation	14.7	9.7	8.7	8.7

The results are the mean of duplicate incubations.

S.E. 7.6%

The results of these four experiments showed
three main facts. (a) The histaminase present in
lung/

lung tissue slowly destroyed the histamine present
in the lung tissue, on incubation of the latter.
(b) Histidine partially inhibited the destruction
of histamine by histaminase.   (c) Benzene increased
the activity of histaminase slightly.   This
phenomenon is similar to the effect of benzene on
histidine decarboxylase, but appeared to be less
marked.

The apparent inhibition of histamine destruction by
histidine, was important because it could either
have been a true inhibition of histaminase or a
formation of some histamine from histidine by lung
tissue. As a result of this observation, the effect
of histidine on the activity of a purified dried
powder of hog's kidney, containing histaminase
activity was determined.   This has already been
described, along with the effect of benzene (page 81).
In both cases, the substances had no inhibitory or
potentiating effects on the activity of the histam-
inase present.   Werle (1942) also showed that l-
histidine had no effect on the activity of
histaminase.

Thus, it did appear possible that lung tissue
might have contained some histidine decarboxylase,
and/

and it was as a result of these observations that
rabbit lung tissue was used in the following three
sets of experiments. In these experiments, lung
tissue was incubated with histidine after removal
of the tissue histamine already present, in order
to try and show the production of histamine from
histidine by a histaminase rich tissue.

b. Incubation of histamine rich
tissue with histidine after removal of the tissue
histamine by long incubation in Tyrode's solution.

Rabbit lung tissue was incubated in buffered
Tyrode's solution (pH 8.2, short range paper) for
7 hours at 38°C. in the presence of benzene. During
this time the histamine present in the tissues was
destroyed by histaminase also present in the tissues.
One sample was tested for histamine in order to
determine how much histamine was present before
incubation with histidine. Histidine was added
to the mixtures which were incubated for a further
3 hours at 38°. Incubation mixtures were made up
in duplicate as shown in Table 28.

TABLE 28/

TABLE 28

Composition of incubation mixtures for 7 hours incubation in a buffered Tyrode's solution followed by a further 3 hours incubation after the addition of histidine.

Minced lung tissue. Wet weight. g.	Tyrode's solution ml	M/5 Na₂HPO₄ solution ml	Water ml	Benzene (1 drop) mg	Histidine soln added after 7 hr incubation mg
0.5	4	2	1	22	-
0.5	4	2	1	22	15
0.5	4	2	1	22	150

In a repeat experiment, an extract of rabbit lung tissue, (prepared as described for a rabbit kidney extract, page 50) obtained by grinding 1 gm tissue in 8 mls Tyrode's solution + 3 mls M/5 Na₂ HPO₄ solution and centrifuging, was used. The extract was allowed to stand at 38° for 6 hours. 6 mls extract were then incubated in duplicate for 18 hours at 38° with 1 ml histidine solution containing 15, 60 or 150 mg histidine, and one drop of benzene. 6 mls of extract were tested for their histamine content, without incubation, with histidine. The results of these two experiments are given in Table 29.

TABLE 29/

TABLE 29

Histamine found in mixtures after double incubation.   First incubation of lung tissue in buffered Tyrode's solution for 7 hours or lung tissue extract for 6 hours followed by a second incubation of 3 and 18 hours respectively with histidine.

	μg histamine/gm tissue
1st experiment	
Histamine at end of first incubation.	A trace.
15 mg histidine in second incubation.	Not detectable 0.02.
150 mg histidine in second incubation.	Not detectable 0.02.
2nd experiment	
Histamine at end of first incubation.	Not detectable.
15 mg histidine in second incubation.	Not detectable.
60 mg histidine in second incubation.	A trace. 0.01
150 mg histidine in second incubation.	A trace. 0.01
Extract. No incubation.	10.2

Each result is the mean of duplicate incubations.

In two further experiments, using the same incubation mixtures as given in Table 28, minced

lung/

lung tissue was incubated for 3 hours at 38°, with
and without benzene and tested for histamine.
Samples which had been incubated for 3 hours without
benzene were then incubated 3 hours at 38° without
histidine, with 15 mg histidine and with 15 mg
histidine + one drop (20 mg) benzene + 0.1 ml 10^{-3} M
aminoguanidine solution.   The results of the two
experiments are given in Table 30.

The results of three of the last four experi-
ments showed that rabbit lung tissue was incapable of
forming histamine from histidine.   In the first
experiment (Table 30) there was a definite increase
in the histamine content of the mixtures containing
aminoguanidine and benzene over the histamine present
before histidine aminoguanidine and benzene were
added, while in the mixtures containing histidine
alone there was no apparent destruction of histamine,
as occurred in mixtures which did not contain
histidine.   There was thus a suggestion that
histamine was formed by lung tissue, but this was
not substantiated by any of the other three
experiments.

TABLE 30/

TABLE 30

Histamine found in mixtures after double incubation. First incubation of lung tissue in buffered Tyrode's solution with and without benzene, followed by a second incubation with histidine, and with histidine, benzene and aminoguanidine.

	μg histamine	
	1st Expt.	2nd Expt.
Histamine in mixture after first incubation.	8.2	5.2
Histamine in mixture after first incubation with benzene present.	8.2	3.1
No histidine in second incubation.	6.1	2.2
15 mg " " "	8.4	2.7
15 mg histidine + benzene aminoguanidine in second incubation.	11.7	4.9
Histamine content of incubation mixture prior to incubation.	13.6	7.3

Each result is the mean of duplicate incubations.   S.E. 4.9%.

Figure IV

The electrodialysis cell

ELECTRODIALYSIS CELL
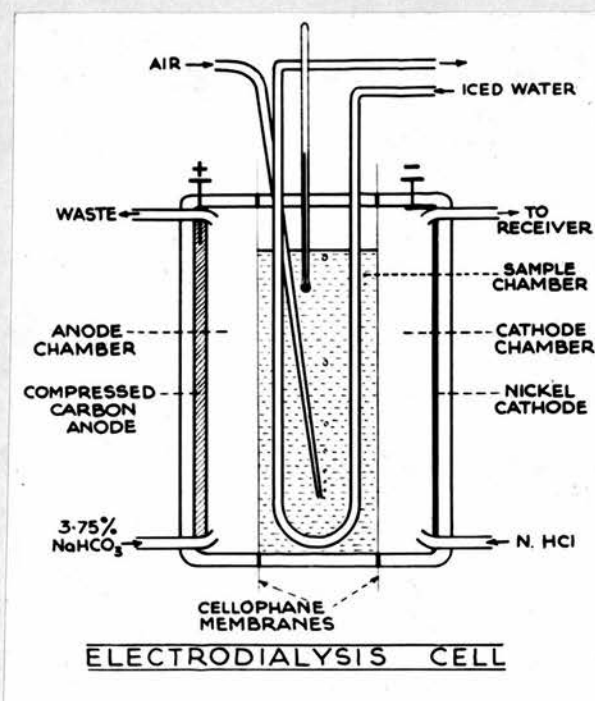
c.   Incubation of electrodialysed histamine rich tissue with histidine.

Rabbit lung mince, in buffered Tyrode's solution, was electrodialysed in a special cell at 38° until no further histamine passed to the cathode, where it was collected.   Histidine was then added, while the cell was still running, and histamine tested for at the cathode.   This was repeated in a second experiment.   A further experiment was performed in duplicate in which the lung tissue was electrodialysed until no further histamine was released, the contents of the cell chamber were then transferred to conical flasks and incubated for 3 hours at 38°C after which they were tested for histamine in the normal manner (page 24).

The cell used was a modification of that described by Thorpe and McGregor (1933).

The cell consisted of three perspex compartments, having smoothly ground faces so that they fitted together to give water-tight joints (Figure IV).   One compartment formed the cathode chamber, of about 7 ml volume, having a nickel cathode at one end.   A second compartment formed the anode chamber, of about 7 ml volume, having a compressed carbon/

carbon anode at one end.   Fitting between these
two compartments was the sample chamber of 20 ml
volume.   Sheets of cellophane were inserted
between the faces, which were greased with
petroleum jelly.   The cell was held together
in a rigid clamp.

The cathode chamber was perfused with 1 N
HCl at a rate of about 0.75 mls/minute. This kept
the cathode chamber acid, preventing destruction of
histamine by the Na^+ ions passing to the cathode
and giving a highly alkaline solution. A little
neutral red indicator was added to the acid so that
any shift of pH above 7 could be detected and the
rate of perfusion accordingly increased.   Excessive
perfusion rates were avoided, since this would
result in lowering the concentration of any histamine
present, and accordingly make it more difficult to
detect.

The anode was perfused with 3.75% $NaHCO_3$
solution at a rate of 5 mls/minute, providing a
ready alkaline carrier for the chlorine released
at the anode.

As the current caused a considerable heating
of the cell, the sample chamber was maintained at
38°C. by passing ice water through a U- tube inserted
into/

into the fluid, the fluid being stirred by a stream
of fine bubbles issuing from a fine jet.

4 gms of the minced lung tissue were placed
in the centre chamber and 12 mls Tyrode's solution
+ 2 mls M/5 Na_2HPO_4 added.   The cell was connected
to the D.C. mains supply (220-230 volts) in series
with two carbon filament lamps arranged in parallel.
This gave a current of 1.2 amps and a potential
difference across the plates of the cell of 20 volts.

The pH of the centre chamber was kept at
pH 8 by the addition of solid sodium bicarbonate,
when necessary, and by keeping the cathode perfus-
ate as slow as possible.

Aliquots of the cathode perfusate (Table 31)
were collected, neutralised to neutral red indi-
cator with solid sodium bicarbonate, and diluted as
many times as possible, but still leaving the
histamine concentration assayable, before assaying
for histamine on the guinea-pig's ileum preparation,
in order to reduce the tonicity of the solution.
After $1\frac{1}{2}$ hours dialysis 30 mg histidine mono-
hydrochloride in 1 ml water (neutralised to uni-
versal indicator paper) were added to the sample
compartment and further samples of the cathode
perfusate collected.

The/

The whole experiment was repeated with a
second sample of rabbit lung tissue. The results
are given in Table 31. The histamine content of
the tissue was obtained by boiling some fresh
tissue in buffered Tyrode's solution, with N HCl,
neutralising and assaying for histamine.

The results showed that a very large percent-
age of the histamine in the tissue was lost, for
only 8.6 µg and 8.7 µg histamine were obtained by
electrodialysis out of a possible 41.2 µg and 34.8µg
respectively. This could have been due to the ions
present in the sample chamber, especially chlorine
ions, and probably some histaminase activity would
be present at the start of electrodialysis.

The whole experiment was repeated using
rabbit kidney tissue mince. No histamine was
detectable before or after the addition of histidine.
This demonstrated that the method was unsuitable, as
the kidney tissue, when incubated separately with
histidine was capable of forming 3.2 µg histamine in
one hour.

TABLE 31/

TABLE 31

Histamine content of the cathode perfusate from the electrodialysis of rabbit lung tissue, before and after the addition of histidine.

Cathode perfusate (Time from start of dialysis). mins.	1st EXPERIMENT		2nd EXPERIMENT	
	Volume of perfusate ml	μg histamine in perfusate	Volume of perfusate ml	μg histamine in perfusate
0-5	4	0.75		
5-10	4	0.95		
10-30	27	2.26	24	4.32
30-45	14	1.53		
45-60	12	1.42	28	3.36
60-75	14	1.33	26	0.94
75-90	17	0.4	17.5	0.5
90-100			5.9	not detected
100-120	27	0.1		
15 mg Histidine added				
0-5	4.0	not detected		
5-10	3.7	"	7.6	not detected
10-15	3.9	"		
15-20	4.1	"	7.4	"
20-25	4.4	"		
25-30	4.1	"	9.2	"
30-45	3.9	"		
45-60	4.2	"	6.0	"

Rabbit lung tissue (fresh)   10.3μg/gm   8.7 μg/gm

Two experiments were performed in which 18 mls rabbit lung extract (10 g tissue in 80 mls 0.9% saline) were placed in the centre chamber.   After 105 minutes electrodialysis/

electrodialysis the samples were removed and incu-
bated with histidine and $M/5$ Na_2HPO_4 solution for 3
hours at $38^\circ C$. The incubation mixtures were tested
for histamine in the normal manner (page 24).

The experiment was repeated using a rabbit
kidney extract (4.7g/23.5 mls 0.9% saline).

The results of these three experiments are
given in Table 32.

TABLE 32/

TABLE 32

Histamine content of rabbit lung and kidney tissue and histamine formed by electrodialysed rabbit lung tissue and rabbit kidney tissue, when incubated with histidine.

	1st EXPERIMENT		2nd EXPERIMENT	
	Volume of perfusate ml	µg histamine in perfusate	Volume of perfusate ml	µg histamine in perfusate
Rabbit lung extract				
5 min. perfusate	3.4	1.63	4.3	0.3
10 " "	3.9	3.12	4.1	2.1
30 " "	20.0	11.2	29.0	5.6
90 " "	56.0	not detected	61.0	not detected
105 " "	10.5	" "	10.0	" "
Rabbit kidney extract				
5 min. perfusate	4.3	not detected		
10 " "	4.7	" "		
30 " "	21.0	" "		
90 " "	53.0	" "		
105 " "	9.7	" "		

3 hour Incubation after electrodialysis

		µg hista/mine/g.		µg histamine in mixture
Rabbit lung extract No histidine in incubation mixture 30 mg " "		not detected "		not detected "
Rabbit kidney extract No histidine in incubation mixture. 30 mg " "		0.01 7.4		
Fresh rabbit kidney extract No electrodialysis		8.3		

The/

The results for the rabbit kidney extract
showed that, within experimental limits, electro-
dialysis had no action in inhibiting histidine de-
carboxylase.   Thus if the lung tissue had contained
any histidine decarboxylase it would be expected
that histamine should have been detectable in the
incubation mixture.   This was not necessarily the
case as some histaminase activity remained in the
centre chamber sample even after long electro-
dialysis, and this could destroy any histamine
formed in the supernatant.

d. Incubation of histamine rich
tissue with histidine after treatment with a hista-
mine liberating substance.

In this experiment, the histamine in the lung
tissue from a rabbit was released by a histamine
liberator - octylamine or decylamine according to
the method of Mongar and Schild (1953). The tissue
was filtered and then washed with Tyrode's solution
and incubated with histidine.   The filtrate contain-
ing the histamine liberator was also incubated with
histidine.   The histamine content was determined
after 3 hours incubation.

4 gm minced rabbit lung were incubated for
one/

one hour at $38^{\circ}C$ with 60 mls 10^{-3} M decylamine in Tyrode's solution. The mixture was filtered, and the paper and residue washed twice with 0.9% saline. 1.5 gm residual tissue was incubated with 16 mls Tyrode's solution and 8 mls $M/5$ Na_2HPO_4 in the presence and absence of 100 mg histidine. 15 ml of filtrate were incubated for 3 hours at 38° in the presence and absence of 100 mg histidine. An aliquot of the filtrate was tested for histamine without incubation.

The whole experiment was repeated for 10^{-3}M octylamine. In the incubations with histidine, one drop (20 mg) benzene was added to each incubation mixture. The results of these two experiments are given in Table 33.

TABLE 33

Histamine released from histamine rich tissue by a histamine liberator and histamine formation by tissue treated with a histamine liberator.

	Total histamine µg	
	Decylamine	Octylamine
Filtrate after treatment of the tissue with histamine liberator.	31.8	29.6
Filtrate incubated for 3 hours with histidine.	21.4	22.6
Histamine liberator treated tissue incubated without histidine.	not detected	not detected
Histamine liberator treated tissue incubated with histidine.	not detected	not detected

From these results, no histamine appeared
to be formed when the histamine liberator treated
tissue was incubated with histidine.   The hista-
mine liberator released all the histamine from the
tissue in 10^{-3} M solution.

The loss of histamine from the filtrate on
incubation could be due to histaminase passing into
this filtrate from the tissue, during incubation
with the histamine liberator.

The whole of this last experiment was
repeated using minced rabbit kidney tissue.   After
treatment with octylamine, but not after decylamine,
benzene (20 mg) was added to the incubation
mixtures containing histidine. Some of the kidney
tissue was incubated, in the first incubation with
Tyrode's solution, in place of the solution contain-
ing the histamine liberator, after which it was
filtered.   The residue and filtrate were then incu-
bated with histidine.   Some fresh kidney tissue
was incubated directly with histidine in the
presence and absence of benzene to determine the
full activity per gramme of original tissue.   The
results are given in Table 34.

TABLE 34/

TABLE 34

Histamine formed when rabbit kidney tissue was
incubated with histidine, before and after treatment with
a histamine liberating substance

	µg histamine/gm			
	Decyl-amine	Octyl-amine	TYRODE'S SOLUTION absence of benzene	presence of benzene
Fresh minced kidney tissue. Direct incubation with histidine.			8.5 absence of benzene	35.4 presence of benzene
Filtrate from first incubation: a. incubation in absence of histidine	0.1	0.18	0.1	0.1
b. " " " presence	0.86	3.2	2.7	11.8
Minced kidney tissue (after 1st incubation either with Tyrode's solution or solution of histamine liberator)	1.3	7.1	4.7	19.2

Each result is the mean of duplicate incubations.

S.E. 5.2%

The following facts appeared from the results.

(1) Approximately 30% of the histidine decarboxylase
activity passed into the supernatant, from the tissue,

during/

during the first incubation.   (2)   There was an
inhibition of approximately 70% of the histidine
decarboxylase activity of the minced tissue after
treatment with 10^{-3} M decylamine or octylamine. This
inhibition was also found in the supernatant includ-
ing the histamine liberator.

Since both decylamine and octylamine
inhibited the action of histidine decarboxylase by
as much as 70% no further research was carried out
involving the use of histamine liberating substances.

e.   Distribution of histidine
decarboxylase in animal tissues.

It has been demonstrated (Part II, page 73)
that aminoguanidine in a 10^{-4} M concentration
inhibits the histamine destroying activity of the
cat's kidney, while it has no inhibitory action on
the histidine decarboxylase activity of rabbit
kidneys.   It has also been shown that benzene pro-
duces a 4-7-fold increase in the amount of histamine
formed by rabbit kidney tissue from histidine.

In the following experiments, many tissues,
from the several animals available in the laboratory,
were incubated in a buffered Tyrode's solution con-
taining histidine in the presence of 10^{-4} M amino-
guanidine and 20 mg benzene.   This gave the optimal

conditions/

conditions for the production of histamine from histidine, if any histidine decarboxylase was present in the tissue.

At the same time, the histamine content of each tissue was determined by boiling the tissue in Tyrode's solution, acidified to pH 5.5, without previous incubation. Further, the histaminase activity of each tissue was determined in a simple manner, by incubating the tissue with added histamine and measuring the histamine left after 3 hours incubation at 38°C. In assessing the results, allowance was made for the histamine estimated to be present in the tissue at the start of the incubation.

The animals, except the cats and dogs, were killed by a blow on the head, followed by cutting of both carotids. The cats and dogs were killed by an overdose of chloroform-ether mixture and bled from the carotids. The tissues were removed as quickly as possible and immersed in 0.9% saline. Any extraneous tissue was dissected off. Stomach and intestines were washed through with saline, cut open and rinsed with saline, until all signs of their contents were removed. The kidneys were removed from their capsules and the pelvis cut away. The liver/

liver, lung, pancreas and skeletal muscle (thigh) were minced directly. All tissues, except a few mouse and rat tissues, were minced in a Latapie hand mincing machine, and aliquots of the minced tissues used in the incubations. Where it was found that there was insufficient tissue for mincing, the tissue was snipped into small parts by scissors.

The rat tissue results were obtained in each experiment from the pooled tissue of two rats, while to obtain the figures for mice, the tissues of three mice were pooled.

In most cases 0.5 g tissue was present in the incubation mixtures. Otherwise, as much tissue as was available was used in the experiment. In all cases the results, for the histamine content of the tissue, and the histamine produced from histidine by the histidine decarboxylase present in the tissue, were expressed as $\mu g/g$ tissue. In such calculations allowance was made for the water content of the tissue and for the amount of tissue used.

Incubation mixtures of each tissue were made up as shown in Table 35.

TABLE 35/

TABLE 35

Incubation mixtures used to determine the histamine content and the histaminase and histidine decarboxylase activities of a selection of tissues from various animals.

Incubation mixture No.	Tyrode's soln. ml	M/5 Na₂HPO₄ soln. ml	Histidine soln. 15mg/ml ml	Histamine soln. ml	Water ml	Benzene 20 mg	10 M amino-guanidine soln. 0.1 ml
A	5	3	1	-	-	+	0.1 ml
B	5	3	-	1	-	-	-
C	5	3	-	-	1	-	-

Mixture A was incubated for 3 hours at 38°C.
The results for the histamine content of the mixture
after incubation is shown in Table 36, column 7.
Subtraction of the amount of histamine estimated to
be present originally in the tissue (Column 1) gives
the amount of histamine formed from the histidine
(Column 8) and is a measure of the histidine
decarboxylase activity of the tissue. The histidine
decarboxylase activity of the tissue is expressed
arbitrarily in Column 9.

Mixture C was acidified to pH 5.5 (short
range paper) with 1 N HCl. The mixture was brought
to the boil, cooled, filtered, and the filtrate
assayed for histamine. These results are given in
Table 36, Column 1, and are a measure of the
histamine present in the tissue at the start of the
experiment.

Mixture B was incubated for 3 hours at 38°.
The histamine solution added was either 20 μg/ml or
10 μg/ml. In Table 36, Column 3, the amount of
histamine present in the incubation mixture at the
start of the incubation is given. It is the sum
of the histamine added and the histamine estimated
to be present in the tissue at the start of the
incubation/

incubation (Column 1).   The histamine content of
the mixture found after incubation is given in
Colmn 4.   The histamine destroyed by the tissue
in the 3 hours incubation period (Column 3 -
Column 4) appears in Column 5, and is a measure
of the histaminase activity of the tissue.   This
has been expressed arbitrarily in Column 6.

In Table 36, Columns 2, 6 and 9 give the
histamine content, histaminase activity and
histidine decarboxylase activity respectively,
expressed in arbitrary units.

TABLE 36/

TABLE 36

The histamine content and the distribution of histaminase and histidine decarboxylase activities of a selection of tissues of various animals.

Animal and tissue	µg histamine/g tissue	Histamine content of tissue arbitrary	µg histamine in incubation mixture	µg histamine after incubation with histamine	µg histamine destroyed 5 g tissue	histaminase activity arbitrary	histamine/g after incubation with histidine and benzene	µg histamine/g formed	Histidine decarboxylase activity.
	1	2	3	4	5	6	7	8	9
Rabbit									
Kidney 1	0.5	-	20	20.5	nil	-	114	113.5	++++
2	0.1	-	10	10.2	nil	-	23.1	23.0	++
Liver 1	1.3	-	20.5	20.8	nil	-	18.0	16.7	++
2	0.5	-	20.2	19.8	0.4	-	15.3	14.8	++
Duodenum 1	5.8	+	12.9	10.5	2.4	+	4.9	nil	-
2	13.5	++	17.8	7.8	10.6	++	14.6	1.1	-
Ileum 1	1.5	+	10.75	9.75	1.0	-	3.4	1.9	+
2	1.6	+	10.8	nil	10.8	++	8.2	6.6	++
Stomach									
Outer layer 1	nil	-	10.0	8.5	1.5	-	nil	nil	-
2	nil	-	20.0	20.1	0.1	-	nil	nil	-
Inner cardiac	5.7	+	12.3	12.3	nil	-	7.7	2.0	+
Inner pyloric	3.1	+	11.6	12.0	nil	-	17.4	14.1	++
Whole inner layer	10.8	++	14.3	14.1	0.2	-	11.5	0.7	-
Colon 1	2.9	+	11.5	9.8	1.3	-	2.5	nil	-
2	3.6	+	11.8	10.2	1.2	-	3.8	nil	-
Caecum 1	12.4	++	16.2	16.2	nil	-	11.0	nil	-
2	10.8	++	25.4	25.4	nil	-	10.5	nil	-
Spleen 1	33.6	+++	26.8	26.2	0.6	-	33.0	nil	-
2	37.3	+++	38.7	38.4	0.3	-	36.2	nil	-
Skeletal muscle 1	nil	-	20.0	19.7	0.3	-	1.1	1.1	-
2	nil	-	10.0	10.1	nil	-	nil	nil	-
Pancreas	nil	-	10.0	8.1	1.9	+	0.2	nil	-
Skin	nil	-	10.0	9.7	0.3	-	nil	nil	-
Lung 1	18.0	++	19.0	15.8	3.2	+	18.0	nil	-
2	7.3	+	13.7	9.6	4.1	+	7.1	nil	-

Guinea-pig/

TABLE 36 Contd: 117

Animal and tissue		μg histamine/g tissue 1	Histamine content of tissue arbitrary 2	μg histamine in incubation mixture 3	μg histamine after incubation with histamine 4	μg histamine destroyed/ 5 g tissue 5	histaminase activity arbitrary 6	histamine/g after incubation with histidine and benzene 7	μg histamine/g formed 8	Histidine decarboxylase activity. 9
Guinea-pig										
Kidney	1	1.8	-	10.9	9.9	0.9	-	82.0	80.8	+++
	2	0.2	-	10.1	10.2	nil	-	26.4	26.2	++
Liver	1	2.4	+	11.4	5.2	6.2	+	27.0	24.6	++
	2	0.3	-	10.1	10.3	nil	-	12.2	11.9	++
Duodenum	1	6.3	+	13.1	8.4	4.7	+	83.0	76.7	+++
	2	17.0	++	28.5	17.0	8.5	+	32.0	15.0	++
Ileum	1	5.0	+	12.6	6.5	6.0	+	36.0	31.0	+++
	2	12.8	++	16.4	3.6	12.8	++	32.7	19.9	++
Colon	1	2.0	+	11.0	10.4	0.6	-	3.5	1.5	-
	2	3.1	+	12.0	11.6	0.4	-	3.8	1.1	-
Whole stomach	1	2.7	+	11.3	11.2	0.1	-	8.2	5.5	+
	2	10.5	++	25.1	25.0	0.1	-	15.0	4.5	+
Lung		10.9	++	15.4	14.0	1.4	-	11.5	0.6	-
Skeletal muscle		nil	-	10.6	10.8	nil	-	0.7	0.7	-
Rat										
Kidney	1	nil	-	10.0	8.9	1.1	-	3.6	3.6	+
	2	nil	-	20.0	20.2	nil	-	6.4	6.4	+
Liver	1	nil	-	10.0	9.2	0.8	-	0.61	0.6	-
	2	1.0	-	10.5	10.5	nil	-	7.2	6.2	+
Duodenum	1	4.6	+	12.3	2.8	9.5	+	7.6	3.0	+
	2	15.8	++	27.9	2.4	25.5	++	19.2	3.4	+
Ileum	1	11.2	++	15.6	0.5	15.1	++	10.7	nil	-
	2	26.2	++	33.1	1.0	32.1	+++	26.2	nil	-
Colon	1	9.0	++	14.5	5.9	9.6	+	9.9	0.9	-
	2	4.3	+	12.1	8.4	3.7	+	4.1	nil	-
Whole stomach	1	37.2	+++	28.6	30.6	nil	-	79.0	42.0	+++
	2	28.9	++	34.4	28.0	6.4	+	34.0	5.1	+
Lung		15.7	++	24.7	24.1	0.6	-	15.7	nil	-

Mouse/

TABLE 36 Contd:

Animal and tissue	μg histamine/ g tissue	Histamine content of tissue arbitrary	μg histamine in incubation mixture	μg histamine after incubation with histamine	μg histamine destroyed/ 5 g tissue	Histaminase activity arbitrary	histamine/ g after incubation with histidine and benzene	μg histamine/ g formed	Histidine decarboxylase activity
	1	2	3	4	5	6	7	8	9
Mouse									
Kidney 1	4.0	+	12.0	12.0	nil	-	291.0	287.0	+++++
2	nil	-	20.0	20.6	nil	-	66.7	66.7	+++
Liver 1	nil	-	10.0	10.6	nil	-	5.1	5.1	+
2	nil	-	20.0	20.8	nil	-	7.1	7.1	+
Duoden- um 1	nil	-	10.0	6.2	3.8	+	1.3	1.3	+
" 2	1.5	+	20.5	0.2	20	++	5.7	4.2	+
Ileum 1	nil	-	10.0	10.0	nil	-	nil	nil	-
2	1.1	+	20.5	nil	20	++	1.0	nil	-
Whole stomach 1	4.5	+	11.8	10.9	0.9	-	9.1	4.6	+
" 2	10.0	++	23.3	24.3	nil	-	22.3	12.3	+++
Colon	nil	-	10.0	6.2	3.8	+	nil	nil	-
Lung	nil	-	10.0	8.8	1.2	-	nil	nil	-
Spleen	nil	-	10.0	9.0	1.0	-	nil	nil	-
Cat									
Kidney 1	nil	-	100	0.1	170	++++	nil	nil	-
2	nil	-	100	nil	250	+++++	nil	nil	-
Liver 1	nil	-	20	20.6	nil	-	0.1	nil	-
2	nil	-	20	19.0	1.0	-	nil	nil	-
Duoden- um 1	40.0	+++	90.0	20.0	70.0	+++	36	nil	-
2	31.0	+++	85.0	49.5	35.5	+++	29	nil	
Ileum 1	35.5	+++	37.8	0.9	36.9	+++	no results		
2	15.0	++	77.5	21.0	56.5	+++	13.0	nil	-
Stomach - cardiac 1	15.0	++	27.5	24.0	3.5	+	14.3	nil	-
2	16.0	++	28.0	32.0	nil	-	14.8	nil	-
Pyloric 1	10.0	++	25.0	25.5	nil	-	10.7	nil	-
2	13.0	++	26.5	26.0	0.5	-	13.0	nil	-
Lung 1	29.0	++	34.5	32.0	2.5	+	27.9	nil	-
2	8.8	+	24.4	20.0	4.4	+	8.3	nil	-
Pancreas	1.0	-	20.5	15.3	5.2	+	0.8	nil	-
2	nil	-	20.0	18.0	2.0	+	nil	nil	-
Spleen	nil	-	20.0	19.0	1.0	-	1.4	1.4	-

Dog/

TABLE 36 Contd: 119

Animal and tissue	μg histamine/ g tissue	Histamine content of tissue arbitrary	μg histamine in incubation mixture	μg histamine after incubation with histamine	μg histamine destroyed/ 5 g tissue	Histaminase activity arbitrary	histamine/ g after incubation with histidine and benzene	μg histamine/ g formed	Histidine decarboxylase activity
	1	2	3	4	5	6	7	8	9
Dog									
Kidney 1	nil	–	100.0	nil	170	++++	nil	nil	–
2	nil	–	100.0	nil	250	+++++	nil	nil	–
Liver 1	14.0	++	27.0	15	12	++	10.0	nil	–
2	7.8	+	23.0	19.9	3.9	+	6.9	nil	–
Duoden- um 1	30.0	+++	35.0	5.3	29.7	++	26.0	nil	–
2	37.1	+++	38.6	4.1	34.5	+++	32.1	nil	–
Ileum 1	25.0	++	32.5	8.5	24.0	++	24.0	nil	–
2	33.1	+++	35.6	4.6	31.0	+++	33.3	nil	–
Stomach:									
Cardiac 1	42.0	+++	41.0	44.1	nil	–	40.0	nil	–
2	30.1	+++	35.0	34.0	nil	–	29.8	nil	–
Pyloric 1	24.0	++	32.0	36.0	nil	–	23.0	nil	–
2	18.0	++	29.0	30.3	nil	–	18.7	0.7	–
Lung 1	84.0	+++	62.0	66.0	6.0	+	79.0	nil	–
2	61.0	+++	51.0	47.6	3.4	+	58.1	nil	–

100 μg histamine added to 0.3 g tissue

100 μg histamine added to 0.2 g tissue

70 μg histamine added to 0.4 g tissue

To facilitate comparisons of the tissues of
various animals, the histamine contents, the
histaminase activities and the histidine decarboxy-
lase activities, expressed in a semi-quantitative
fashion are summarized in Table 37.

Key to TABLE 37/

TABLE 37/

KEY TO TABLE 37

	Histamine content.	Histaminase activity.	Histidine decarboxylase activity.
	µg histamine/g	µg histamine destroyed/0.5 g	µg histamine formed/g
- non-significant	0.0-2.0	0.0-2.0	0.0-2.0
+	2.0-10	2.0-10	2.0-10
++	10-30	10-30	10-30
+++	30-100	30-100	30-100
++++	100-250	100-250	100-250
+++++	250	250	250

TABLE 37

A summary of the histamine content and the distribution of the histaminase and histidine decarboxylase activities of selected tissues from various animals, expressed semi-quantitatively.

Tissue / ANIMAL	Kidney	Liver	Whole stomach	Stomach outer layer	Stomach inner cardiac	Stomach inner pyloric	Duodenum	Ileum	Colon	Caecum	Pancreas	Lung	Spleen	Skeletal muscle	Skin
					Mucosal – muscularis layers										
Histidine decarboxylase															
Dog	-	-			-	-	-	-				-			
Cat	-	-	-		-	-	-	-			-	-	-		
Rabbit	+++	++	+	-	+	++	+	+	-	-	-	-	-		-
Guinea-pig	+++	++	+				+++	++	-			-			
Rat	+	+	++				+	-				-			
Mouse	++++	+	++				+	-	-			-	-		
Histaminase															
Dog	+++++	+			-	-	+++	+++				+			
Cat	+++++	-	-		-	-	+++	+++		-	+	+	-		
Rabbit	-	-	-	-	-		++	+	+		+	+	-	-	-
Guinea-pig	-	-	-			-	+	+	-			+		-	
Rat	-	-	-				++	+++	+			-		-	
Mouse	-	-	-				+	++	+			-	-		
Histamine															
Dog	-	+			++	++	+++	+++				+++			
Cat	-	-	++		+++	++	+++	+++			-	+++	-		
Rabbit	-	-	++	-	+	+	++	+	+	++	-	++	+++	-	-
Guinea-pig	-	-	+				++	+	+			++	-		
Rat	-	-	++++				+	++	+			++			
Mouse	-	-	+				+	-	-			-	-		

PART IV

Effect of organic solvents on the decarboxylation of histidine by mammalian tissue preparations.

During some earlier work, (page 65) in which some diamines were being tested as possible potentiators of histidine decarboxylase, one drop of chloroform was added to the incubation mixtures during long incubations in order to prevent the growth of bacteria. It was found that the presence of this chloroform increased the production of histamine from histidine by rabbit kidney tissue by 3-4-fold. This unexpected phenomenon has been investigated further.

First a long series of organic chemicals, both aliphatic and aromatic, were tested. As many different chemical compounds as were obtainable in the laboratory, were tried, to see if there was any co-relation between the chemical structure and their potentiating action. During these tests, it was found that benzene gave the greatest potentiation of/

of all the chemicals tested. It was subsequently
used in most of the further tests. The solu-
bilities of several of the more active compounds
were noted in order to ascertain if the effect
was a phase effect. In a side experiment, benzene
was shaken with a saline solution containing hista-
mine, to determine the distribution of histamine
between saline and benzene.

Since the most active potentiators of the
production of histamine were to be found among the
aromatic solvents, the possibility arose that the
enzyme was fat soluble, and was more readily released
into solution when the compound was added. It might
therefore be expected that detergents would also
exert a potentiating effect. Accordingly, two
detergents were tested. "Teepol XL" B.D.H.
(Lauryl sulphate) an anionic detergent, and
Cetrimide (Cetyl trimethylammonium bromide) a
cationic detergent. These were incubated with
rabbit kidney tissue and histidine and compared with
the results of incubations in which the compound was
absent. However, using concentrations of the
detergents rising to levels, which produced an
inhibitory action on the gut during assay, no
potentiating/

potentiating effect was noticed.

A second possibility was that the increased production of histamine was due to the compound preventing the growth and activity of some bacterium which was normally destroying some of the histamine, immediately it was formed, or preventing the formation of histamine. Thus some incubations were carried out in which the effects of two antibiotics, aureomycin and chloramphenicol, and two sulphonamides, sulphathiazole and phthalyl-sulphathiazole, on the production of histamine, were tested. These had no potentiating effect, in fact phthalylsulphathiazole definitely inhibited the histidine decarboxylase activity.

In order to test whether the benzene had actually to be present during the incubation or if it was increasing the solubilization of the enzyme, some experiments involving freeze-drying, were carried out. In these experiments, rabbit kidney tissue was incubated with and without benzene in a buffered Tyrode's solution for two hours without histidine. Each mixture was freeze-dried, re-dissolved in water, and again incubated with and without benzene in the presence of added histidine. This would have determined if benzene had to be present/

present during the actual incubation to produce an
increase in the histamine production.   The results
of this experiment indicated that pretreatment of
the tissue preparation with benzene failed to in-
crease the histamine yield from histidine, which
was obtained when benzene was present in the incu-
bation mixture with histidine.

The benzene potentiation effect was still
found to persist when benzene was added to the
supernatant from a tissue extract subjected to a
force of 90,000   g.   Such a gravitational force
would result in the removal of all particulate cell
constituents.

a. Effect of chloroform on
histidine decarboxylase activity.

Incubation mixtures were made up in duplicate
as in Table 38.   Each one was incubated for 3 hours
at 38°C.

TABLE 38/

TABLE 38

Incubation mixtures to show the effect of chloroform on the production of histamine by rabbit kidney tissue from histidine.

Test No.	Minced rabbit kidney g	Tyrode's solution ml	m/5 Na₂HPO₄ solution ml	Histidine solution ml	Chloroform
Test	0.4	4	2	1	1 drop
C₁	0.4	4	2	1	-
C₂	0.4	4	2	-	1 drop
C₃	-	4	2	1	1 drop

After incubation, each mixture had the missing quantity added. Then each mixture was tested for histamine. The results of 4 experiments are given in Table 39.

TABLE 39

The increased production of histamine by rabbit kidney tissue from histidine in the presence of chloroform.

Experiment No.	μg histamine in incubation mixture/g tissue				
	Normal Incubation C_1	Incubation with chloroform Test	Tissue blank C_2	Solution blank C_3	Ratio Test/normal
1	6.6	19.7	0.1	nil	3.0
2	10.8	34.4	0.2	nil	3.2
3	1.5	6.8	0.1	nil	4.3
4	3.2	11.0	0.1	nil	3.5

Each result is the mean of duplicate incubations. S.E. 2.6%

The results show a 3-4-fold increase in the
production of histamine from histidine by rabbit
kidney tissue in the presence of chloroform, when
compared with similar incubations in the absence of
chloroform. The results of incubation mixture C_2
show that it is not a release of histamine from the
tissue, and mixture C_3 shows that it is not an
interaction of the reagents. The results of the
normal incubation (C_1) demonstrate the large
individual variation between rabbits of the
histidine decarboxylase content of the kidneys.

b. The effect of benzene and other
organic solvents on the production of histamine
from histidine.

To find out if the increased production of histamine
from histidine by rabbit kidney tissue in the pres-
ence of chloroform, was an effect peculiar to chloro-
form alone, or if it was shown by other organic
solvents, a number of other organic solvents were
tested.

Incubation mixtures consisted of 0.4 g minced
rabbit kidney tissue in 4 ml Tyrode's solution, 2 mls
$M/5$ Na_2HPO_4 solution and 1 ml histidine solution
(15mg/

(15 mg/ml), together with, in (a) one drop of the
organic solvent before the incubation: in (b) one
drop of the organic solvent after the incubation,
and in (c) no addition of organic solvent. Each
incubation was for 3 hours at 38°C. If the sub-
stance potentiated or inhibited the decarboxylation
of histidine this would be seen as a difference in
the results of (a) and (b). If the substance
affected the gut, it would be seen as a difference
in the results of (b) and (c).

During the second experiment it was found that
benzene gave a substantially greater effect on the
incubation than chloroform did. Thus in all subse-
quent experiments the chloroform and benzene effects
were compared with each other as well as with other
test substances. The results of these experiments
with chloroform and benzene are given in a separate
Table 40. As benzene proved to be the most active
potentiator of histidine decarboxylase activity,
it was used in several subsequent experiments, and
the results of these experiments are also included.

TABLE 40/

The increased formation of histamine produced by rabbit kidney tissue from histidine, in the presence of chloroform or benzene.

TABLE 40

Expt no.	CHLOROFORM μg histamine formed/gm			BENZENE μg histamine formed/g			Ratio benzene chloro-form.
	control chloroform after incubation	test chloroform before incubation	Ratio test control	control benzene after incubation	test benzene before incubation	Ratio test control	
1	2.2	9.0	4.1	2.2	27.6	12.5	3.1
2	13.5	36.4	2.7	13.5	99.6	7.4	2.7
3	7.2	24.9	3.4	7.4	56.9	7.9	2.3
4	0.25	0.99	4.0	0.25	10.0	40.0	10.0
5	2.2	5.5	2.5	2.2	6.2	6.2	2.5
6				1.8	12.6	7.0	
7				1.2	15.4	12.5	
8				1.25	6.7	4.7	
9				31.2	114.0	3.8	
10				3.0	27.0	9.1	
11				4.5	24.2	5.3	
12				13.2	88.6	6.7	
13				3.3	14.8	4.5	

Each result is the mean of duplicate incubations. S.E. 3.0%

The results of the histamine assays of the
incubation mixtures to which chloroform or benzene
had not been added at any stage, were the same as
the results obtained for those in which the chloro-
form or benzene was added after incubation.

The results showed that benzene increased the
decarboxylation of histidine from 4-12-fold with
one exceptional result of 40-fold.   In five tests,
benzene was found to have at least 2-3 times as much
effect as chloroform.

In Table 41 the results of several more
organic solvents are given.   Each result is com-
pared with the histamine yield in the absence of
the organic solvent and with the histamine yield
obtained with the same tissue preparation in the
presence of benzene and chloroform.

TABLE 41/

The increased production of histamine formed by minced rabbit kidney tissue from histidine in the presence of several organic solvents compared with that produced in the presence of chloroform and benzene.

TABLE 41

SOLVENT	μg histamine formed/g tissue			Ratio HO/HA	Ratio for Chloroform	Ratio for benzene	Ratio Solvent/Benzene
	Control 2 Solvent absent	Control 1 Solvent added after incubation HA	Test Solvent present during incubation HO				
Pyridine	13.6	13.6	109.0	8.0	2.7	7.4	1.10
Toluene	2.2	1.8	19.7	9.0	4.1	12.5	0.72
Chlorobenzene	7.2	6.9	36.8	5.1	3.5	7.9	0.65
Light Petroleum B:P: 80°-100°							
Cyclohexane	2.2	2.4	13.4	6.1	4.1	12.5	0.48
Bromoform	13.6	12.7	37.4	2.8	2.7	7.4	0.37
Nitrobenzene	9.9	9.7	36.0	3.7	4.3	—	
Aniline	7.2	6.3	17.9	2.5	3.5	7.9	0.32
Diethyl ether	7.2	7.1	15.6	2.2	3.5	7.9	0.28
Carbon tetrachloride	2.2	1.9	6.3	2.9	4.1	12.5	0.23
Acetone	9.9	9.9	28.9	2.9	4.3	—	
Cyclohexone	2.2	2.2	3.4	1.5	4.1	12.5	0.12
Tetrachloroethane	2.1	2.1	2.2	1.0	2.5	6.2	0.15
n-amylalcohol	13.6	12.7	15.0	1.1	2.7	7.4	0.15
n-butylalcohol	8.5	8.7	8.8	1.0	3.1	7.1	0.13
Tetralin	7.2	7.9	6.3	0.87	3.5	7.9	0.11
Ethyl acetate	13.6	13.6	2.3	0.17	2.7	7.4	0.02
Methyliodide	2.1	2.3	0.4	—	2.5	6.2	—
Quinoline	2.1	0.5	nil	—	2.5	6.2	—
Ethyl malonate	13.6	13.6	no assay	—	2.7	7.4	—

Each result is the mean of duplicate incubations.   S.E. 2.1%

From these results it appeared that benzene and pyridine showed the greatest activity in potentiating the formation of histamine from histidine by rabbit kidney tissue, while toluene, chlorobenzene, light petroleum B.P.80^0-100^0 and cyclohexane showed activity greater or as great as chloroform. As there was little difference between benzene and pyridine, benzene was used in further experiments to study this potentiation, for it was easier to obtain and keep a pure sample of benzene than of pyridine.

The solubilities of chloroform, benzene and pyridine in water are 10 mg/ml at 15^0C, 0.82 mg/ml at 22^0C. and totally miscible in all concentrations respectively. As the concentrations of them in the incubation mixtures at 38^0C was 2.2 mg/ml, 2.0 mg/ml and 1.8 mg/ml respectively, chloroform and pyridine must have been totally soluble, while benzene could have been in two phases, however, it seemed unlikely that the effect was due to any phase effects. (Later experiments (page 142) with smaller quantities of benzene appeared to confirm this). However, the distribution of histamine between a tissue extract and benzene was tested in the following experiment.

c./

        c. Partition of histamine
between a tissue extract and benzene.

In this experiment, a mixture of histamine,
a tissue extract and buffer was shaken with benzene,
and the histamine in the aqueous and benzene layers
determined. The procedure was as follows.

Two incubation mixtures, were each shaken with
2 mls benzene in a mechanical shaker at 18°C for one
hour. The incubation mixtures were: (1) 1 ml hista-
mine solution (10μg/ ml) + 5 mls Tyrode's solution+
1 ml M/5 Na₂HPO₄ solution + 2 mls A.R. benzene.
(2) 1 ml histamine solution + 5 mls Tyrode's
extract of rabbit kidney 1 gm tissue/5mls (prepared
as described on page 50) + 1 ml M/5 Na₂HPO₄ solution
+ 2 mls A.R. benzene.

After shaking and centrifuging, 3 mls of the
aqueous layer were removed, normal acid added drop-
wise to pH 5.5 (short range paper) and the mixture
brought to the boil, simmered for one minute to
remove any benzene, cooled, filtered and the hista-
mine content of the filtrate determined. One ml
of the benzene layer was removed and one ml N HCl
added. The mixture was evaporated to dryness in
a/

a water-bath under reduced pressure and the residue
taken up in 2 mls distilled water, neutralised to
pH 5.5 (short range paper) with solid $NaHCO_3$,
brought to the boil, cooled, filtered and assayed
for histamine.

A third sample (3) consisting of 1 ml
histamine solution ($10\mu g/ml$) + 5 mls Tyrode's
extract + 1 ml Na_2HPO_4 solution was adjusted to pH
5.5 (short range paper) brought to the boil, cooled,
filtered and assayed for histamine.   All samples
were done in duplicate.   The results are given in
Table 42.

TABLE 42

The distribution of histamine between a salt
solution and benzene, when a salt solution of hista-
mine was shaken with benzene.

Test mixture	μg histamine found per total volume of layer	
	1st Experiment	2nd Experiment
1. Aqueous layer	9.8	9.6
Benzene layer	nil	nil
2. Aqueous layer	9.2	9.6
Benzene layer	nil	nil
3. Aqueous layer	9.8	9.8

Each result is the mean of duplicate incu-
bations.   S.E. 1.4%.

The/

The results show that no histamine passes into the benzene layer. One possible explanation of the benzene effect on the production of histamine, was that as the histamine was formed it passed into the benzene layer and so more histamine was formed - mass action effect. This explanation is ruled out by the results of this experiment, and also by the results of the previous experiment (page 134) in which it was shown that the benzene was totally in solution and that two layers should not be formed.

d. The effect of the concentration of chloroform, benzene and pyridine on the decarboxylation of histidine.

Rabbit kidney tissue extract was incubated with histidine in the presence of varying concentrations of benzene, chloroform and pyridine, in order to measure the potentiation of histidine decarboxylase by these concentrations of the organic solvent and to determine the minimum amount of organic solvent required to give the potentiation and also to determine the concentration necessary to provide the maximal effect.

The concentration of chloroform was increased by 2, 3 and 4-fold. Incubation mixtures were made up/

up as for the incubation mixtures 1, 2 and 3 in the
experiment to show the effect of chloroform on
histidine decarboxylase (page 63, Table 15).
Additional incubation mixtures were included by
adding 2, 3 or 4 drops of chloroform. Each incu-
bation mixture was set up in duplicate.

After incubation for 3 hours at 38°, the
histamine contents of the incubation mixtures were
determined, Table 43.

TABLE 43

Effect of varying concentrations of chloro-
form on the production of histamine from histidine
by rabbit kidney tissue extracts.

CHLOROFORM ADDED			μg HISTAMINE/gm TISSUE			
Drops	Weight mg	Molarity in incu- bation mixtures	Control 1 Chloroform added after incubation	Test added before incubation	Control 2 Chloro- form added before. No histi dine.	Ratio Test/ Control 1
1	21.4	0.024M	2.6	11.8	0.1	4.5
2	42.7	0.048M	2.5	11.9	0.1	4.6
3	64.6	0.072M	2.7	5.8	0.1	2.2
4	86.6	0.096M	2.8	3.4	0.1	1.2

Each result is the mean of duplicate incubations. S.E. 1.2%

The/

The effect of chloroform appeared to be
maximal between 0.024M-0.048M. Further increase
in concentration decreased the activation but this
was still evident to a slight extent with a concen-
tration 0.096M.

Chloroform saturation of water at 15°C is
0.084M.

The results of a similar experiment in which
benzene was added in place of chloroform are given
in Table 44.

TABLE 44

Effect of varying concentrations of benzene on
the production of histamine by rabbit kidney tissue
extracts.

BENZENE ADDED			µg HISTAMINE/gm TISSUE			
Drops	Weight mg	Concen- tration mg/ml	Control 1 Benzene added after incubation	Test Benzene added before incubation	Control 2 Benzene added before No histi- dine.	Ratio Test/ Control 1
1	20.4	2.4	1.8	12.6	0.1	7.0
2	40.7	4.8	1.8	12.3	0.1	6.8
3	60.5	7.2	1.7	12.8	0.1	7.5
4	80.4	9.6	1.6	12.2	0.1	7.6
1 ml	880.0	10.6	1.8	12.0	0.1	6.7

Each result is the mean of duplicate incubations. S.E. 1.0%.

In/

In Table 31 the concentrations greater than 0.82 mg/ml are purely arbitrary, as this is the concentration of benzene in a saturated aqueous solution at 18°C. The results suggest that the effect of benzene reaches a maximum around the concentration of benzene in water at which the water is fully saturated. Any increase above this value has no effect, potentiatory or inhibitory on the increased production of histamine.

In the next experiment, benzene was again tested, but over a far greater range of concentrations, varying from 0.068 mg/ml-10 mg/ml. The very small concentrations of benzene were obtained by adding benzene to tyrode and taking aliquots of this tyrode. The benzene solutions were made up as follows.

Tyrode's solution A.   - 1 drop (25 mg) benzene in 100 mls Tyrode's solution = 0.25 mg/g.

Tyrode's solution B.   - 1 drop (25 mg) benzene in 20 mls Tyrode's solution = 1.25 mg/ml.

Each Tyrode's solution was shaken in a stoppered flask for 45 minutes at 38°C before use.

A rabbit kidney extract was used as a source of/

of the enzyme.   It was prepared as described on
page 50.   2 mls of rabbit kidney extract were
incubated with 1 ml histidine solution (15 mg/ml)
and 2 mls $M/5$ Na_2HPO_4 solution, and 5 mls of Tyrode's
solution.   For concentrations of benzene less than
0.81 mg/ml, 5 mls of Tyrode's solution, made by
varying amounts of either Tyrode's solution A or
Tyrode's solution B made up to 5 mls with pure
Tyrode's solution, were used, to give a total
incubation mixture volume of 10 mls.   For benzene
concentrations greater than 0.81 mg/ml the benzene
was weighed into the flask and 5 mls of pure
Tyrode's solution added.   A blank incubation con-
tained no benzene but 5 mls of pure Tyrode's
solution.   Each mixture was incubated for 3 hours
at $38^{\circ}C$, then the histamine in the mixtures was
determined.   The results are given in Table 45 and
shown graphically in Figure V.

In a second experiment, a very small
quantity and a very large quantity of benzene were
used.   The results for this experiment are also
given in Table 45.

TABLE 45/

TABLE 45

Effect of varying concentrations of benzene on the production of histamine from histidine by rabbit kidney tissue extract.

Benzene added mg/ml	µg histamine per incubation mixture	Ratio	histamine (benzene present) / histamine (benzene absent)
1st Expt.			
No benzene	2.2		-
0.07	6.7		3.0
0.12	8.6		3.9
0.18	12.5		5.7
0.25	14.8		6.7
0.38	18.5		8.4
0.50	17.0		7.8
0.81	21.8		9.8
2.14	18.5		8.4
3.3 (1drop)	21.7		9.8
6.6[+]	18.5		8.4
9.9[+]	18.5		8.4
13.2[+]	18.5		8.4
2nd Expt.			
No benzene	8.8		-
0.025	19.5		2.2
0.05	33.1		3.7
3.2 (1 drop)	46.8		5.3
1 ml[+]	47.6		5.4

[+] Undissolved benzene visible after incubation.

Each result is the mean of duplicate incubations. S.E. 2.0%

The increase in the production of histamine from

histidine/

Figure V
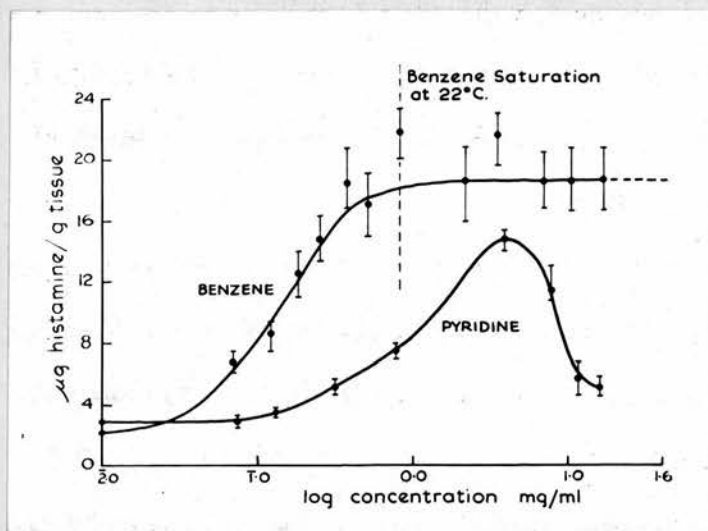
       The relation between the histamine formed and the amount of benzene or pyridine present in the incubation mixtures when rabbit kidney tissue was incubated with histidine.

histidine reached a maximum in the presence of about 0.38-0.81 mg/ml. A saturated solution of benzene in water at 22°C contains 0.81 mg/ml benzene. The addition of amounts of benzene in excess of that required to saturate the solution, did not further increase the histamine yield. Thus it would appear that the maximal production of histamine is achieved when the incubation mixture is saturated with benzene, while incubations of benzene as low as 25µg/ml produced a marked but submaximal effect.

As benzene gave a maximal effect when the incubation mixture was saturated with the solvent, the experiment was repeated using instead of benzene, pyridine, which is soluble in water in all proportions and which had already been found to be an effective potentiator. A Tyrode's solution containing pyridine was made by dissolving 2 drops 77.5 mg pyridine in 50 mls Tyrode's solution. The pyridine was weighed directly into the incubation flask, if the final concentration was to be greater than 3.8 mg/ml. The results are given in Table 46. and shown graphically in Figure V.

TABLE 46/

TABLE 46

Effect of varying concentrations of pyridine on the production of histamine from histidine by rabbit kidney tissue extract.

Pyridine added mg/ml	μg histamine per incubation mixture	Ratio	histamine (pyridine present) / histamine (pyridine absent)
No pyridine	2.9		-
0.077	2.9		1.0
0.155	3.4		1.2
0.31	4.9		1.7
0.78	7.4		2.6
3.8	14.7		5.1
7.7	11.4		4.0
11.6	5.7		1.9
15.5	5.1		1.7
3.8 (after incubation)	2.7		-
3.2 (benzene)	17.4		6.0

Each result is the mean of duplicate incubations.   S.E. 1.5%

The results showed that concentrations of less than about 1 mg/ml pyridine in the incubation mixtures, had no effect, but in concentrations greater than this, the pyridine caused an increase in the production of histamine, the maximum being reached at about 3.8 mg/ml.   The maximum yield/

yield of histamine was similar to that obtained
in the presence of excess benzene. If the pyridine
concentration was increased beyond 3-8 mg/ml, the
potentiating effect showed a rapid decline, though
it was still noticeable with a concentration of
15.5 mg/ml.

e. The effect of chloroform and
benzene on the production of histamine from histidine
for various tissue states and the effect of benzene
on tissue extracts subjected to ultra high-speed
centrifugation.

Experiments were performed in which either
chloroform or benzene was incubated with histidine
and the histidine decarboxylase present in various
tissue states, i.e. sliced tissue, minced tissue and
tissue homogenates. As the increased production of
histamine was seen in the tissue supernatants,
prepared as on page 50, it was believed that the
enzyme might be contained in either the mito-
chondria or the microsomes or that the enzyme might
be in pure solution. If the enzyme was in either
of these cell bodies, the effect of benzene or other
organic solvent might be explained, by some inter-
ference at the cell face, allowing better enzyme-
substrate/

substrate contact. If the enzyme was in pure solution, some other explanation would have to be sought.

In order to test this, the extract prepared from rabbit kidney tissue was subjected to differential centrifugation, according to Schneeder and Hogeboom (1950). Each supernatant and each residue were incubated with histidine, with and without benzene.

This ultra centrifugation was also used to study the distribution of the enzyme, as the results for the incubations with histidine, would show in which fractions the enzyme was present.

In the first experiment, sliced rabbit kidney (page 27) minced rabbit kidney (page 50) or an extract of rabbit kidney (page 50) was incubated in Tyrode's solution for 3 hours at 38°, together with histidine, in the presence and absence of chloroform. The incubation mixtures were prepared, in duplicate as indicated in Table 47.

TABLE 47/

TABLE 47

Incubation mixtures to determine if the potentiation of histamine formation was independant of the tissue form.

Incubation No.	Kidney tissue	Tyrode's solution	M/5 Na₂ HPO₄ soln.	Histidine soln. 15mg/ml	Test Chloroform before	Control after incubation
		ml	ml	ml	22mg	22mg
1	0.4 g sliced tissue	4	2	1	+	-
2	0.4 g sliced tissue	4	2	1	-	+
3	0.4 g minced tissue	4	2	1	+	-
4	0.4 g minced tissue	4	2	1	-	+
5	2 mls extract 0.4 g tissue	2	2	1	+	-
6	2 mls extract 0.4 g tissue	2	2	1	-	+

The results of the histamine assays of these incubation mixtures are given in Table 48.

TABLE 48/

TABLE 48

The histamine formed by slices, a mince and an extract of rabbit kidney tissue when incubated with histidine in the presence and absence of chloroform.

Tissue	μg Histamine in incubation/ gm tissue		Ratio $\frac{test}{control}$
	Control	Test	
Sliced tissue	4.9	17.8	3.6
Minced tissue	5.8	21.4	3.6
Tissue extract	6.5	22.5	2.5

Each result is the mean of duplicate incubations.   S.E. 1.5%.

These results show that the effect of chloroform is the same for sliced and minced tissue and for tissue extracts.

Two similar experiments, using benzene in place of chloroform, were also carried out, with the results which are shown in Table 49.

TABLE 49

The histamine formed by slices, a mince and an extract of rabbit kidney tissue when incubated with histidine, in the presence and absence of benzene.

| TISSUE | µg Histamine formed/g tissue | | RATIO $\frac{test}{control}$ |
	Control	Test	
Expt I			
Kidney slice	3.2	16.2	5.0
Kidney mince	3.3	15.8	4.8
Kidney extract	2.3	11.2	4.9
Expt II			
Kidney slice	0.9	6.7	7.4
Kidney mince	1.2	6.7	5.3
Kidney extract	1.2	7.8	6.7

Each result is the mean of duplicate incubations.   S.E. 1.5%.

These results (Tables 48 and 49) showed that the/

the increased production of histamine from histidine
by rabbit kidney tissue in the presence of chloro-
form or benzene was independant of the tissue form .
As this effect was seen in a kidney extract, sub-
jected to 2,500× g, the effect must have been either
as the cell constituents left in suspension, or a
pure solution effect.   To study this problem and
to study the distribution of the enzyme in the
tissue cell components, experiments involving
differential centrifugation were carried out.
Extracts of rabbit kidney tissue were prepared and
then subjected to increasing gravitational forces.
After each centrifugation, aliquots of the super-
natants and the residues were incubated with histi-
dine in the presence and absence of benzene.   The
detailed procedure was as follows: 0.45 gms of
minced rabbit kidney tissue was incubated with and
without benzene, to ascertain the activity of the
enzyme in the fresh tissue.   6.2 g minced kidney
tissue were homogenised with 28 mls 0.25M sucrose
solution at 0° in a Potter-Elvehjeim homogeniser,
cooled in iced water.   The homogenate was centri-
fuged at 2,500×g for 15 minutes at room temperature
(16°).   The enzyme activity of 2 mls supernatant
and/

and 0.45 g residue was determined by incubation with histidine in the presence and absence of benzene. The rest of the supernatant was divided into two 12 ml portions and centrifuged at 33,000 × g for 45 minutes at -2°. Each residue was incubated with histidine, one in the presence of benzene, the other in the absence of benzene. An aliquot of each supernatant (4 mls) was incubated with histidine, one with benzene, the other without. The remainder of each supernatant (6 mls) was centrifuged at 90,000 × g$^+$ for 60 minutes at 7°C. Each residue was incubated with histidine, one in the presence of benzene and the other in the absence of benzene. Each supernatant was divided into two parts, each of which was incubated with histidine, one in the presence of benzene and the other in the absence of benzene.

Incubation mixtures were made up as follows: the volume of each supernatant was made up to 4 mls with 0.25M sucrose solution, or 4 mls of 0.25M sucrose solution was added to the tissue mince or residue/

$^+$At 90,000 × g centrifugation particles 35 μg will precipitate. Particles of 100 μg will be precipitated in 7-8 minutes.

residue of each centrifugation.   Each of those
incubation mixtures were completed by adding 2 mls
M/5 disodium hydrogen phosphate solution and 1 ml of
histidine solution (15 mg/ml).   Benzene was added
as required.   Each incubation was for 3 hours at
38°.

In a separate experiment it was shown that the
production of histamine from histidine by rabbit
kidney tissue was unaffected by changing from
Tyrode's solution-buffer mixture to an 0.25M sucrose
solution-buffer mixture.

A duplicate experiment, using kidney tissue from
another rabbit was also carried out.   The results
of these two experiments are given in Table 50.

Before the ultra-centrifuge was available three
experiments were performed in which the differential
centrifugation was carried as far as centrifugation
at 37,000 x g.   The experimental procedure was
essentially the same as that already described above.
These results are also given in Table 50.

TABLE 50/

TABLE 50

Histamine formed by rabbit kidney tissue extracts subjected to varying gravitational forces and by the residues from each centrifugation.

The results are expressed in µg histamine formed/gm original tissue.

Minced tissue	1st CENTRIFUGATION	2nd	3rd	Presence of benzene
	2,500g	33,000g 37,000g	90,000g	
7.0	6.4 0.8	6.0 0.9	6.2 0.2	-
31.3	27.6 5.2	37.9 2.1	31.2 0.4	+
3.1	4.8 nil	3.2 not done	3.5 0.2	-
17.5	16.5 1.3	not done 0.2	16.2 0.3	+
2.9	1.9 0.02	1.5 0.01		-
13.3	13.1 0.04	10.9 0.01		+
2.0	2.2 0.16	3.0 0.02		-
19.0	18.5 0.21	18.9 0.03		+

4.1/

TABLE 50 Contd.

Minced tissue	1st 2,500g	2nd CENTRIFUGATION 33,000g 37,000g	3rd 90,000g	Presence of benzene
4.1 ⟶	3.8 ⟶ 0.4 ⟶	3.8 0.03		-
29.5 ⟶	28.0 ⟶ 0.9 ⟶	26.6 0.06		+

(Note: In each pair of figures, the upper figure represents the supernatant, and the lower one, the residue).

These results are summarized (Table 52) and discussed along with the results for the intracellular distribution of histaminase and histamine.

High speed centrifugation of histaminase-rich tissue.

In parallel with the experiments involving differential centrifugation of rabbit kidney tissue homogenates, a few experiments were carried out involving differential centrifugation of histaminase rich tissues, in order to determine in which particular cell body the histaminase was contained, and to determine if it was possible to separate the enzyme histidine decarboxylase from histaminase by this method. At the same time, the distribution of the histamine contained in the histaminase-rich tissue, was also determined. Rabbit lung tissue was used for these experiments, as this tissue was already known to contain both histamine and histaminase.

The histaminase activity was determined by measuring the amount of added histamine destroyed in a given time when the tissue preparation was incubated with histamine, allowance being made for the histamine already present in the tissue. The histaminase activity and the histamine content of the minced tissue were determined, then again for each residue/

residue and supernatant of a tissue extract, prepared exactly as for rabbit kidney tissue, by homogenising minced lung tissue in a Potter-Elvehjeim homogeniser with 0.25M sucrose, and then centrifuging at 2,500 X g for 30 minutes followed by further centrifugation at 37,000 X g for 60 minutes.

To each sample for incubation 2 mls saline together with 2 mls M/3 phosphate buffer pH 7.2 and 1 ml histamine solution (10 μg) were added. Each mixture was incubated for $1^{\frac{1}{2}}$ hours at 38° after which the histamine content of each mixture was determined as described on page 24.

To obtain the histamine content of the tissue or homogenate, 0.5 ml N HCl was added, the mixture boiled for one minute, cooled and the pH then adjusted to 5.5 (short range paper) with solid NaHCO$_3$. The mixture was filtered and the filtrate assayed for histamine.

The experiment was repeated using a lung sample from another rabbit. The results of these two experiments are given in Table 51.

TABLE 51/

TABLE 51

The histaminase activity and the histamine content of rabbit lung extracts subjected to varying gravitational forces and of the residues from each centrifugation.

Minced tissue	1st CENTRIFUGATION 2,500g		2nd 37,000g

Histaminase activity. (µg histamine destroyed/1 hour/ gm tissue).

3.2	⟶	3.4 nil	⟶	2.7 nil
5.8	⟶	6.0 nil	⟶	3.5 nil

Histamine content. (µg histamine found/gm tissue)

4.1	⟶	4.0 0.4	⟶	1.2 2.47
10.3	⟶	9.0 0.3	⟶	2.0 9.64

(Note: In each pair of figures, the upper figure represents the supernatant and the lower one, the residue).

In a further experiment, involving a final centrifugation at 90,000×g which was carried out in order to determine if the histaminase was in pure solution/

solution or in the microsomes, it was found that
the tissue sample contained no histaminase activity
and no histamine.   Thus no results were obtained.

Schneider and Hogeboom (1950) stated that for
rat liver homogenates made up in 0.25M sucrose, 10
minutes centrifugation at 700 × g sedimented all
the nuclei, red blood cells and unbroken liver
cells, increasing the centrifugation to 10 minutes
at 5,000 × g sedimented the mitochondria, while 60
minutes centrifugation at 57,000 × g sedimented the
microsomes.   Thus in these present experiments,
it has been assumed that the slow speed residue con-
tained the unbroken cells, cell nuclei and any
blood cells which may have been present.   The high
speed residue was assumed to contain the mitochon-
dria, while the ultra high speed residue contained
the microsomes.   The ultra high speed supernatant
was believed to contain only substances in pure
solution and a lipid layer at the top, containing
secretory granules (Schneider and Hogeboom, 1951).

On this basis, the intracellular distribution
of the histaminase activity and the histamine
content in rabbit lung tissue together with the
intracellular distribution of histidine decarboxylase
in rabbit kidney tissue have been summarized in
Table 52./

TABLE 52

The intracellular distributions of histidine decarboxylase in rabbit kidney tissue and histaminase and histamine in rabbit lung tissue.

Part of cell	Histidine decarboxylase activity.	histaminase activity	Histamine
Cell debris	5.8%	nil	6.3%
	(0 - 16.6)	0.2%	(2.9 - 9.7)
Mitochondria	2.1%	nil	76.9%
	(0 - 12.8)	0.2%	(60.2 - 93.6)
Microsomes) and) solution)	96.2%	72.4%	24.3%
	(52 - 150)	(60.4-84.4)	(19.4 - 29.3)
Total	104.1% 10 Experiments	72.4% 2 Experiments	107.5% 2 Experiments

Histidine decarboxylase activity.

4 Experiments

Microsomes and)
solution.   )   99.0 ──────→ Microsomes   0.4%
                                        ↘ Supernatant   98.5%

From these results, it appeared that the enzyme histidine decarboxylase was readily soluble in either Tyrode's/

Tyrode's solution or 0.25M sucrose solution, and was thus found in the supernatant solution after centrifugation at 90,000 × g. If the enzyme was contained in any particular cell body, it diffused out very readily, and it was impossible to show the presence of the enzyme in any specific fraction.

The same results were obtained for the histaminase activity of rabbit lung tissue. Although it was unfortunately impossible to demonstrate whether the histaminase was in the microsomes or the supernatant solution, it was shown that most of the activity was to be found in the supernatant obtained after centrifugation at 37,000×g, which contained only the microsomes.

However, the results showed that 62-93% of the histamine, contained in rabbit lung tissue, was located in the mitochondria of the cells. This observation confirms the work of Copenhaver, Nagler and Goth (1953) and Hagen (1954) on rat and dog livers respectively. Further, the observation shows that the mitochondria were not destroyed in the experiments described here.

As the ability of benzene to potentiate the decarboxylation of histidine was still observed in the ultra high speed supernatant, any explanation involving/

involving the passage of the enzyme or the sub-
strate through some cell membrane, the transport
being enhanced in some way by benzene seems unten-
able.

f. Incubation time and the effect
of chloroform and benzene on the production of
histamine from histidine by rabbit kidney tissue.

The effect of increasing the
time of incubation on the production of histamine
has been described already (page 44).   When these
experiments were done, the effect of chloroform on
the production of histamine was not known, and it
was added to incubations involving an incubation
time greater than 8 hours.   In these experiments,
the production of histamine from histidine by rabbit
kidney tissue, was compared with the production in
the presence of chloroform or benzene.   In the
first experiment, the production of histamine was
measured every ½ hour up to 3 hours.   In the second
experiment, the production of histamine was measured
at various times up to 48 hours.

Three sets of incubation mixtures were made up,
as described for standard incubations, page 50.
The first set was incubated as described, and was
called/

Figure VI
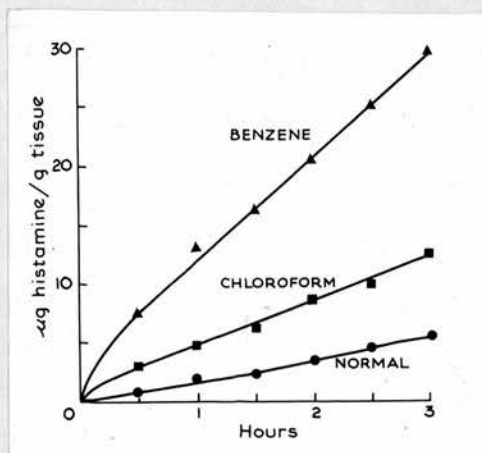
The relation between the histamine formed
and the time of incubation, up to 3 hours when
rabbit kidney tissue was incubated with histi-
dine in the presence and absence of benzene or
chloroform.

●————● Normal incubation.   No benzene or
                chloroform present.

■————■ Chloroform present in incubation
                mixtures.

▲————▲ Benzene present in incubation
                mixtures.

called the normal incubation. To each mixture
in the second set of mixtures one drop of chloroform
(20 mg) was added. To each mixture in the third
set of incubation mixtures, one drop of benzene
(22 mg) was added. Each incubation mixture was
incubated at 38°C. Two samples from each set
of incubations were removed at $\frac{1}{2}$ hourly intervals
from the incubator to give incubation times of
$\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$ and 3 hours and the histamine
content determined. The results of this experiment
are given in Table 53.

TABLE 53

The relation between the histamine formed from
histidine by rabbit kidney tissue in the presence or
absence of chloroform or benzene and the time of incu-
bation during an incubation of 3 hours duration.

Incu-bation time	µg histamine found/gm tissue			
	Normal incubation	Chloroform added	Benzene added	No histidine
(hrs)				
$\frac{1}{2}$	0.6	2.8	7.8	
1	1.8	4.7	13.0	0.1
$1\frac{1}{2}$	2.2	7.4	16.4	
2	3.2	8.5	20.4	0.1
$2\frac{1}{2}$	4.4	10.0	24.9	
3	5.1	12.5	29.8	0.1

Each result is the mean of duplicate incubations.
S.E. 1.7%.

The/

Figure VII
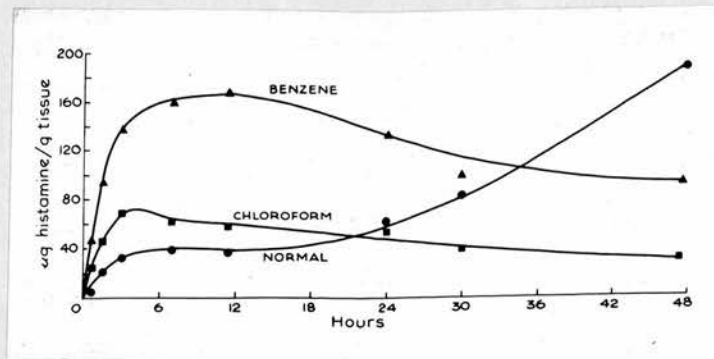
The relation between the histamine formed and the time of incubation, up to 48 hours, when rabbit kidney tissue was incubated with histidine in the presence and absence of benzene or chloroform.

●——● Normal incubation. No benzene or chloroform present.

■——■ Chloroform present in incubation mixtures.

▲——▲ Benzene present in incubation mixtures.

The results from a second similar experiment in which the maximum incubation time was extended to 48 hours are shown in Table 54.

TABLE 54

The relation between the histamine formed from histidine by rabbit kidney tissue in the presence or absence of chloroform or benzene and the time of incubation during an incubation of 48 hours duration.

Incu-bation time. hrs.	μg Histamine found/gm tissue		
	Normal incubation	Chloroform added	Benzene added
$\frac{3}{4}$	4.5	25.7	48.2
$1\frac{1}{2}$	22.0	46.9	94.1
3	33.0	68.4	137.0
7	38.6	60.1	160.0
$11\frac{1}{2}$	36.8	57.3	166.3
24	62.0	51.3	132.0
30	82.0	39.6	98.2
48	182.0	29.8	92.5

Each result is the mean of duplicate incubations. S.E. 1.6%.

The results of these two experiments have been plotted graphically, Figures VI and VII respectively.

As/

As the second experiment involved long incubation times, it was necessary to follow the natural destruction of histamine in Tyrode's solution at pH 8.2 over the same period. Accordingly, three histamine solutions containing 150 μg, 75 μg and 40 μg histamine in 7 mls Tyrode + 3 mls M/5 Na₂HPO₄ buffer pH 8.2 (short range paper) were placed in the incubator. 1 ml of each solution was removed after 6, 14, 21, 30 and 46 hours. From these results, the normal disappearance of histamine from histamine solutions was determined. The results are given in Table 55.

TABLE 55

The normal destruction of histamine in three Tyrode's solutions containing 150 μg, 75 μg and 40μg histamine respectively in Tyrode's solution at pH 8.2 over a period of incubation of 46 hours.

Incubation time. hrs	μg Histamine found/10 mls solution		
	Solution 1	Solution 2	Solution 3
0	150	75	40
6	150	75	42
14	140	70	38.7
21	121	64	31.2
30	106.5	61.5	32.1
46	89	52	28.2

Each result is the mean of duplicate incubations.
S.E. 1.5%.

From/

From the results for the normal disappearance
of histamine from a solution of histamine in Tyrode
it would appear that in the long incubations of
histidine decarboxylase containing tissue the
decrease in the histamine yields from the benzene
and chloroform containing incubation mixtures after
6-11 hours incubation could result from a partial
destruction of histamine.

The results of the long incubations of histidine
decarboxylase containing tissue (Table 41 and 42)
showed that up to 3 hours the amount of histamine
formed had a linear relationship to the time of
incubation, for normal incubations, and for
incubations including chloroform and benzene, only
the slope, that is, the rate of production varied.
This was greatest for benzene, while chloroform was
greater than the normal.   The effect of benzene and
chloroform was apparent even after only $\frac{1}{2}$ hour
incubation.

From the longer incubations, two significant
facts appeared.   (1) Each set of incubations
reached a maximum at about 4-6 hours, after which
the histamine formation apparently ceased, the
histamine already formed then being slowly destroyed
in/

in a very similar fashion to that of the standard histamine solutions (Table 43).   (2) After about 16-20 hours, there is a marked rise in the production of histamine in the normal incubations, which becomes very marked about 40 hours, instead of a fall as seen in the incubation mixtures containing chloroform and benzene.   As these two latter substances would prevent the growth of bacteria, this sudden increase in production, could have been caused by bacterial action.

g. The effect of detergents on the histidine decarboxylase activity.

In order to determine if the effect of benzene on the production of histamine by rabbit kidney tissue was due to a surface action of benzene on the cell surface, to allow a better transferance of either the enzyme or substrate across the lining membrane, rabbit kidney tissue was incubated with two detergents.   This test was carried out before it was known that the potentiating effect of benzene on histamine production was still observable in tissue extracts freed of all particulate cell components by ultra high speed centrifugation.

Two detergents, which were used, were cetrimide (cetyl/

(cetyltrimethyl ammonium bromide), a cationic
detergent, and Teepol XL (B.D.H.), (Lauryl sulphate),
an anionic detergent.

In a series of incubations 0.4 g minced rabbit
kidney tissue was incubated with 4 mls Tyrode's
solution + 2 mls M/5 Na_2HPO_4 solution + 1 ml
histidine solution (15 mg/ml) in the presence and
absence of one drop of detergent solution, exactly
as described for chloroform (Table 38). Incu-
bations were for 3 hours at $38^{\circ}C$. One drop of
detergent was added after incubation to one mixture
to determine if the substance affected the gut.

The detergent solutions were solutions of
cetrimide and Teepol XL containing 200 mg/ml,
20 mg/ml and 2 mg/ml.

The results of the incubations are given in
Table 56.

TABLE 56

Concentration of detergent in incubation mixture. (0.04ml added)	µg Histamine found/gm tissue		
	Detergent added after	Detergent added before	No detergent added
Cetrimide			
1.0 mg/ml	Gut completely inhibited		3.6
0.1 "	3.0	2.4	3.6
0.01 "	3.8	3.6	3.6
Teepol XL			
1.0	Gut completely inhibited		
0.1	1.8	2.0	3.6
0.01	3.8	3.4	3.6

Each result is the mean of duplicate incubations.
S.E. 3.2%.

The results showed that neither detergent, up to concentrations affecting the gut during assay, increased the production of histamine. The results for 20 mg/ml detergent solutions are low, due to gut inhibition, but as the detergent was present before assay, the results for the detergent added before and after incubation were comparable. Here again no potentiating effect was found.

1 drop of 20 mg/ml produced a calculated bath concentration of 5 μg in a 2 ml bath. 4.0 μg of each detergent was added direct to the isolated organ bath. In each case a slight inhibition of the following histamine response was noted, the effect being greater for Teepol XL.

The experiment showed that the effect of benzene was unlikely to be due solely to a surface-active effect on the cell membrane.

h. The effect of two antibiotics and a sulphonamide on the histidine decarboxylase activity.

In order to determine if the effect of benzene on the production of histamine by rabbit kidney tissue was due to a bacteriostatic action, in preventing the/

the growth of a bacterium, normally present, having the power to destroy histamine, rabbit kidney tissue was incubated with two antibiotics and a sulphonamide.

In a series of incubations, 0.4 g minced rabbit kidney tissue was incubated with 4 mls Tyrode's solution + 2 mls $M/5$ Na_2HPO_4 solution + 1 ml histidine solution (15 mg/ml) for 3 hours at $38^\circ C$ and with an aqueous solution or suspension of the drug. Further incubations were carried out in which no drug was present during the incubation, after incubation the drugs were added to some of the mixtures and not to the others. This would show if the drugs had any effect on the gut during the assay. The results are given in Table 57.

TABLE 57/

TABLE 57

Effect of two antibiotics and two sulphonamides on the histidine decarboxylase activity of rabbit kidney tissue.

Drug	Concentration of drug added mg/ml	Volume of drug added ml	Final concentration in mixture mg/ml	μg Histamine/gm tissue		
				Drug absent during incubation	Drug present during incubation	Drug absent altogether
Suspension of chloramphenicol	-	1 drop (0.04)	-	2.9	3.1	3.0
Solution of aureomycin	15.6	1.0	2.1	-	0.4	4.5
	15.6	0.2	0.4	3.0	2.7	4.5
	1.56	1.0	0.2	9.2	8.4	8.8
Solution of sulphathiazole	10.0	0.1	0.14	4.3	3.7	5.8
	1.0	0.1	0.014	5.8	5.4	5.8
	0.1	0.1	0.0014	5.8	5.8	5.8
Solution of phthalyl-sulpha-thiazole	133.0	1.0	17.5	-	0.1	4.5
	133.0	0.2	3.5	1.6	0.2	4.5
	13.3	0.5	0.85	8.7	0.2	8.8
	13.3	0.2	0.34	8.2	0.2	8.8

Each result is the mean of duplicate incubations. S.E. 1.8%.

It is questionable if a bacteriostatic solution was obtained with chloramphenicol, due to its low solubility. Concentrations of aureomycin greater than 0.41 mg/ml inhibited the gut during subsequent assay

but/

but even at this concentration, the production of
histamine was unaffected by the presence of this
drug.

Sulphathiazole in a solution of 0.14 mg/ml in
the incubation mixture, though it showed a slight
inhibitory action on the gut, also showed that there
was no significant difference if a solution of
sulphathiazole was added before or after the incu-
bation.   This was supported by the lower dilutions.
Those three solutions were considered bacterio-
static.

Phthalylsulphathiazole definitely inhibited the
formation of histamine in solutions containing the
drug at a concentration of 0.36 mg/ml or greater.
Concentrations of 3.5 mg/ml and greater, also
inhibited the gut during subsequent assay.

Thus the results showed that a solution,
considered bacteriostatic, did not give any increase
in the production of histamine, and it was therefore
unlikely that the effect due to benzene was due to
inhibition of a histamine destroying bacterium.

i. The/

i. The effect of freeze-drying
rabbit kidney extracts before and after the addition
of benzene and subsequent incubation with histidine.

The effect of benzene on the production of
histamine from histidine by mammalian tissues may
have been due to an action of benzene, in which
more enzyme was brought into solution, and thus
made available for decarboxylation in the presence
of benzene. If this was the case, then the benzene
should only have been needed to be present prior
to or at the start of incubation, after which it
took no further part. In order to determine if this
was true or whether the benzene was necessary during
the period of the incubation to give an increased
formation of histamine, the physiological technique
of freeze-drying the incubation mixture prior to
incubation with histidine was employed.

A rabbit kidney extract was prepared (page 50)
containing 0.25 g wet tissue/ml. The procedure was
as follows:

Into each of six 25 ml conical flasks were
pipetted 3 ml tissue extract, 2 ml M/5 Na_2HPO_4
solution and 2 ml Tyrode's solution. In addition,
one drop (22 mg) benzene was introduced into three
of/

of the mixtures (C. D. and F.).   The flasks were
then stoppered and the mixtures incubated for $2\frac{1}{2}$
hours at 38°C.   Two of the samples containing
benzene (C. and D.) and two to which no benzene
had been added (A. and B.) were freeze-dried (20
hours).   Each resulting residue was dissolved in
7 mls distilled water + 2 ml Tyrode's solution and
1 ml (15 mg) histidine solution added.   One drop
(22 mg) benzene was added to two of the samples
(B. and D.), in one of which (D) benzene was
present during the preliminary incubation.   No
benzene was added to the other two specimens A. and
C.   The mixtures were incubated at 38°C. for 3 hours
and the histamine contents then determined.

To the two original incubation mixtures not
subjected to freeze-drying (E. and F.) were added
2 mls Tyrode's solution and 1 ml (15 mg) histidine
solution and the mixtures maintained at 38° for 3
hours before determining the histamine contents.

The results of two such experiments using the
kidneys from two rabbits are given in Table 58.   In
each case, the figure quoted for the histamine
content of each incubation mixture being the mean
value obtained from duplicate experiments.

TABLE 58/

TABLE 58

Histamine formed by rabbit kidney tissue when incubated with histidine in the presence or absence of benzene and before and after freeze-drying of the tissue in Tyrode's solution.

| Mixture | Benzene | | Total histamine μg | |
	before freeze-drying	after	Expt. 1	Expt. 2
A.	-	-	1.2	0.73
B.	-	+	5.2	9.2
C.	+	-	0.97	0.85
D.	+	+	5.9	10.5
E.	-	(no	1.1	0.82
F.	+	(freeze-(drying	10.8	16.7

Each result is the mean of duplicate incubations.

S.E. 5.6%.

From these results it was seen that benzene must be present during the actual incubation to give an increase in the production of histamine. This was shown by the difference of the results of samples C. and B. and the similarity between C. and A. If the enzyme had been released into solution or been/

been made more readily available to histidine then sample C. should have been equal to B. or A.

There appears to be a slight loss of enzyme activity in the presence of benzene, due to freeze-drying, as is seen by the difference in the results of samples B. or D. and F, particularly in Experiment 1.

PART V

Purification of histidine decarboxylase

Werle and Heitzer (1938) claimed that kaolin
adsorbed histaminase but not histidine decarboxylase,
and that if a solution containing those enzymes was
shaken with kaolin, the histaminase activity could
be removed from the solution.   They further claimed
that the histidine decarboxylase could be adsorbed
by aluminium $C\gamma$ and eluted from this by $M/5$ Na_2HPO_4
solution, and finally precipitated by half satura-
tion of the solution with ammonium sulphate.   By a
combination of these three methods they claimed to
obtain a 35-fold purification of the enzyme in a
kidney extract.

A few experiments were carried out to repeat the
work of Werle, but as the results were not very
promising, this approach to the purification of
histidine decarboxylase was not pursued further.
There was insufficient time to permit an investi-
gation of the possible use of ion exchange resins
to separate the enzyme from tissue extracts.

a./

a. Purification of histidine decarboxylase of kidney extracts by kaolin.

Extracts of rabbit kidney tissue were shaken repeatedly with kaolin and the histidine decarboxylase activity of the extracts before and after each shaking was determined.

An extract of rabbit kidney tissue (E.) was prepared (page 50) by grinding 9 gms of tissue with silver sand and 36 mls tyrode's solution. 2 mls of this extract E. were incubated with histidine, to determine the original histidine decarboxylase activity of the extract.

25 mls of E. were shaken vigorously by hand for 8 minutes with 4 gms kaolin. The supernatant was centrifuged (1,500xg for 10 mins) and the supernatant (E_2) removed. 16 mls E_2 were shaken vigorously for 8 minutes with 2 gms kaolin then centrifuged at 1,500xg for 10 minutes (supernatant - E_3). 11 mls E_3 were shaken vigorously for 8 minutes with 2 gms kaolin and centrifuged at 1,500xg for 10 minutes (supernatant - E_4). 5 mls E_4 were shaken vigorously for 8 minutes with 1 gm kaolin and centrifuged at 1,500xg for 10 minutes (supernatant - E_5).

Incubation/

Incubation mixtures were prepared by adding
2 mls Tyrode's solution + 2 mls M/5 Na_2HPO_4 + 1 ml
histidine solution (15 mg/ml) to 2 mls of each of
the extracts E_1, E_2, E_3, E_4 or E_5).   The mixtures
were incubated for 3 hours at 38°C and the histamine
content of each mixture determined.   Each incu-
bation was done in duplicate.

The experiment was repeated using 16 mls of
rabbit kidney extract (E_1) made by grinding 5 gms
tissue with silver sand and 20 mls Tyrode's
solution, which were shaken for 8 minutes with 2 gms
kaolin and centrifuged (supernatant -E_2).   The
kaolin treatment was repeated as in the first
experiment to give supernatants E_3 and E_4.
Incubation mixtures of the original and the kaolin
extracts were prepared as in the previous experiment.
The results of the two experiments are given in
Table 59.

TABLE 59/

TABLE 59

Histamine formed from histidine by a rabbit kidney extract before and after repeated shaking with kaolin.

Test		µg histamine found/gm tissue.			
		1st Experiment		2nd Experiment	
			% original activity.		% original activity.
Before kaolin	E_1	6.4	-	3.1	-
1st extraction with kaolin	E_2	5.8	91	3.1	100
2nd " "	E_3	4.2	66	2.6	83
3rd " "	E_4	0.7	11	1.3	41
4th " "	E_5	nil	0	-	-

Each result is the mean of duplicate incubations.

S.E. 3.5%.

The results of these two experiments showed that there was no increased activity of the histidine decarboxylase of a rabbit kidney extract after shaking with kaolin. There was in fact, a loss of activity, not significant after a single treatment with/

with kaolin, but rising to 15-30% after a second
extraction and increasing further with repeated
extractions.   It therefore appeared that the
histidine decarboxylase was adsorbed by kaolin on
repeated shaking with it, and was removed from the
solution.

b. Purification of histidine
decarboxylase of rabbit kidney extracts by Celite.

This was a repeat of the two previous experiments,
except that the kaolin was replaced by celite.
29 mls rabbit kidney extract, (9 gms tissue ground
with silver sand and 36 mls Tyrode's solution) was
shaken 8 minutes with 4 gm celite and centrifuged
at 1,500xg for 10 minutes (supernatant - E_2).
14 mls E_2 were shaken 8 minutes with 1.5 gms celite
and centrifuged (supernatant - E_3).   6.5 mls E_3
were shaken for 8 minutes with 0.5 gm celite and
centrifuged (supernatant - E_4).

2 mls of each of E_1, E_2, E_3 or E_4 were incubated
with 2 mls Tyrode's solution + 2 mls M/5 Na_2HPO_4
solution + 1 ml histidine solution 15 mg/ml for 3
hours at 38°C, and the mixtures tested for histamine.
Each incubation was done in duplicate.

In a second experiment, 27 mls of an extract E,
made/

made from 9 gms tissue and 36 mls Tyrode's solution
was treated exactly as in the first experiment.
The results of these two experiments are given in
Table 60.

TABLE 60

Histamine formed from histidine by a rabbit
kidney extract before and after repeated shaking
with celite.

TEST		histamine found			
		1st Experiment		2nd Experiment	
		$\mu g/gm$	% original activity.	$\mu g/gm$	% original activity.
Before Celite	E_1	18.3	-	2.3	-
1st Extraction with celite	E_2	16.9	94	2.5	108
2nd " "	E_3	18.9	103	2.3	100
3rd " "	E_4	17.1	95	2.1	93

Each result is the mean of duplicate incubations.

S.E. 3.3%

The results of these two experiments, show that,
within/

within the limits of experimental error, celite has
no effect, either to potentiate or adsorb the enzyme
histidine decarboxylase.

c. Adsorption of histidine decarboxy-
lase by aluminium oxide.

An extract of rabbit kidney was made by grinding
6.7 gms of minced kidney tissue with 33.5 mls 0.9%
NaCl solution and about 10 gms of silver sand. The
mixture was centrifuged at 2.500 X g, for 10 minutes
and the supernatant removed (Extract E).

20 mls of the extract E were shaken with 4.0 gms
aluminium oxide (Savory and Moore "Standardised
according to Brockmann, for chromatographic analysis")
for 30 minutes in a mechanical shaker. The mixture
was centrifuged at 2,500 X g. The supernatant was
decanted, and filtered to give S_1. The residue was
washed with a little saline, then stirred with 20mls
M/5 Na_2HPO_4 solution and shaken mechanically for
30 minutes. The mixture was centrifuged at 2,500 X
g. The supernatant was decanted and filtered to
give S_2.

Incubation mixtures were made up in duplicate
as follows: 3 mls of E or S_1 were incubated with
3 mls M/5 Na_2HPO_4 solution + 1 ml histidine solution
(15 mg/ml) for 3 hours at 38°C. 3 mls of S_2 were
incubated/

incubated with 3 mls 0.9% saline together with 1 ml (15 mg) histidine solution for 3 hours at 38°. To one of each duplicate sample, 1 drop (22 mg) benzene was added. After incubation, each mixture was tested for histamine.

The whole experiment was repeated using the kidney extract made from the kidneys of a second rabbit. The results of these two experiments are given in Table 61.

TABLE 61

Histamine formed from histidine by a rabbit kidney extract before and after shaking with aluminium oxide and by the eluate after the elution of aluminium oxide with $M/5$ Na_2HPO_4 solution.

PREPARATION		μg histamine found/g orig. tissue			
		1st Expt.		2nd Expt.	
		without benzene	with benzene	without benzene	With benzene
Before aluminium trioxide	E	4.3	26.3	6.4	24.7
Supernatant after shaking with aluminium trioxide	S_1	2.4	17.0	3.8	15.0
Eluate after shaking M/5 Na_2HPO_4 with aluminium trioxide.	S_2	0.87	4.1	1.2	3.9

Each result is the mean of duplicate incubations.

S.E. 4.9%

The/

The results showed that only 40-45% of the
total histidine decarboxylase was adsorbed in
aluminium oxide, and of this, only 31-36% (18-20%
total activity) was eluted by $M/5$ Na_2HPO_4 solution.

Nevertheless, the results showed that the
adsorbed and finally eluted enzyme was still
activated by benzene.

d. Adsorption of histaminase activity
by kaolin.

This experiment was very similar to that already
described for the purification of histidine decarb-
oxylase, except that an extract of rabbit lung
tissue was used, in place of a rabbit kidney extract.
22 mls of a rabbit lung extract E, prepared by
grinding 6 gms rabbit lung mince with 28 mls Tyrode's
solution, were shaken for 8 minutes by hand, with
4 gm kaolin and centrifuged at 1,500×g for 10
minutes (supernatant - E_2).   17 mls E_2 were shaken
8 minutes with 2 gm kaolin and centrifuged (super-
natant - E_3).   12 mls E_3 were shaken 8 minutes
with 2 gm kaolin and centrifuged (supernatant-E_4).
7 mls E_4 were shaken 8 minutes with 1gm kaolin and
centrifuged (supernatant - E_5).

2 mls of each of E_1, E_2, E_3, E_4 or E_5 were incu-
bated in duplicate with 2 mls Tyrode's solution +
2mls/

2 mls M/3 phosphate buffer pH 7.2 + 1 ml histamine solution (20 µg/ml) for 90 minutes at 38°C. After incubation the histamine content of each mixture was determined. The results are given in Table 62.

TABLE 62

Histamine destroyed when a standard solution of histamine (20 µg/ml) was incubated with an extract of rabbit lung tissue, before and after repeated shaking with kaolin.

TEST		µg histamine found per incubation mixture.	µg histamine destroyed per incubation mixture.
Before kaolin	E_1	0.1	20.0
1st extraction with kaolin	E_2	2.6	17.4
2nd " "	E_3	16.6	3.4
3rd " "	E_4	20.8	nil
4th " "	E_5	20.8	nil

Each result is the mean of duplicate incubations. S.E. 2.7%.

The results showed that repeated extraction with kaolin would remove histaminase activity from a solution including the enzyme. Two successive extractions with kaolin remove 75% of the histaminase, the/

the remainder being completely removed by a third
extraction.

e. Adsorption of histaminase by
celite.

This experiment was a repeat of the previous
one except that the kaolin was replaced by celite.
22 mls of a rabbit lung extract E_1 (6 gms lung mince
ground with silver sand and 28 mls Tyrode's solution)
were shaken with 3 gm celite and centrifuged to
give E_2.   8.5 mls E_3 were shaken with 1 g celite
and centrifuged to give E_3.

2 mls of each of E_1, E_2 or E_3 were incubated as
in the previous experiment with 1 ml histamine
solution (10 μg).   Each incubation was done in
duplicate.   The results are given in Table 63.

TABLE 63

Histamine destroyed when a standard solution of
histamine (10 μg/ml) was incubated with an extract
of rabbit lung tissue, before and after repeated
shaking with kaolin.

Rabbit lung extract Text mixture	μg histamine found in mixture	μg histamine destroyed	% histaminase activity left.
Before celite E_1	4.9	5.1	-
1st Extraction with celite E_2	5.4	4.6	92
2nd Extraction with celite E_3	6.0	4.0	80

Each result is the mean of duplicate incubations   S.E.4.8%

Celite did not appear to be very active in adsorbing histaminase from a rabbit lung extract. After a second extraction with celite (E_3) there appeared to be a slight adsorption, but this was still non-significant.   No further experiments were performed with this adsorbent as it did not appear to be very active towards either histidine decarboxylase (Table 60) or histaminase.

f. Adsorption of histaminase by aluminium oxide.

An extract of rabbit lung tissue was made by grinding 5.97 gms of minced lung tissue with 35.8 mls 0.9% NaCl solution and about 10 gms silver sand.   The mixture was centrifuged at 2,500X g for 10 minutes and the supernatant decanted (extract E.).

20 mls of the extract E were shaken vigorously with 4.0 gms aluminium oxide for 30 minutes in a mechanical shaker.   The mixture was centrifuged at 2,500 X g.   The supernatant was decanted and filtered to give S_1.   The residue was washed with a little saline, then stirred with 20 mls M/5 Na_2HPO_4 solution and shaken mechanically for 30 minutes.   The mixture was centrifuged at 2,500 X g. The/

The supernatant was decanted and filtered to give S_2.

Incubation mixtures were made up in duplicate as follows: 3 mls E. or S_1 were incubated with 3 mls M/5 Na_2HPO_4 solution + 1 ml histamine solution (20 μg base/ml). 3 mls S_2 were incubated with 3 mls 0.9% NaCl + 1 ml histamine solution (20 μg base/ml). The pH of each incubation mixture was lowered to pH 7.3 (short range indicator paper) by a dropwise addition of N HCl. Each mixture was incubated for 3 hours at 38°C. The histamine content of each mixture was determined after incubation. The results are given in Table 64.

TABLE 64

Histamine destroyed when a standard solution of histamine (μg/ml) was incubated with an extract of rabbit lung before and after shaking with aluminium oxide and with the eluate after elution of the aluminium oxide with M/5 Na_2HPO_4 solution.

TEST		μg histamine found in mixture	μg histamine destroyed	% histaminase activity left.
Before aluminium trioxide	E	22.1	3.1	-
Supernatant after shaking with aluminium trioxide	S_1	19.6	2.8	90%
Eluate after shaking M/5 Na_2HPO_4 soln with aluminium trioxide	S_2	19.3	0.9	
Blank on 3mls extract E.		5.2 μg		

Each result is the mean of duplicate incubations. S.E. 2.8%.

In a separate experiment 5 µg histamine in 7 mls saline were shaken with aluminium oxide, in the same way as the extract in the preceding experiment. It was found that 2.8µg histamine were adsorbed. On elution with M/5 Na$_2$HPO$_4$ 1.6 µg histamine were eluted off the aluminium oxide. Thus in estimating the above results for the histamine destroyed, this latter has been calculated by subtracting the histamine found from the histamine present at the start. Before shaking with kaolin 5.2 µg histamine were present in the 3 mls extract E together with 20 µg added - total 25.2 µg. On the basis of the second experiment, 3 mls supernatant after shaking with kaolin would have contained 2.4 µg together with 20 µg histamine added - total 22.4. The total eluate would have contained 1.6 µg histamine. Thus 3 mls must have contained 0.2 µg together with 20 µg added histamine - total 20.2 µg.

The results were not very good, but it appeared that some histaminase (about 10%), was adsorbed by the aluminium oxide, off which it was eluted by M/5 Na$_2$HPO$_4$ solution. However, those results were non-significant and no real conclusion can be drawn from them. No further experiments were carried out, due/

due to the poor activity of the adsorbent, either towards histidine decarboxylase or histaminase.

None of the adsorbents tried (kaolin, celite aluminium oxide) were found to be effective differential adsorbents of histidine decarboxylase or histaminase, and the results did not support the claims made by Werle and Heitzer (1938). Thus this attempt at separation of the enzyme was not continued any further.

g. Purification of histidine decarboxylase by precipitation by half saturation with ammonium sulphate.

6.8 gms minced rabbit kidney were homogenised in a Potter-Elvehjem homogeniser with 18 mls 0.9% NaCl solution. The homogenate was allowed to stand for 30 minutes at 38°C in an incubator to allow the maximum solution of the enzyme. It was then centrifuged at 2,500 × g to precipitate all whole cells, broken cells and nuclei. The cloudy supernatant was filtered twice, on Whatman No.41 filter papers - Extract E.

13 mls saturated ammonium sulphate solution were added to 13 mls of extract E. The mixture was shaken vigorously and allowed to stand for 10 minutes. A white/

white precipitate of the proteins settled to the bottom and was filtered off. The filtrate (F_1) was a clear solution.

The protein residue was weighed and found to be about 2.5 gms when in a very wet state. This was taken up in 10 mls 0.9% saline to give a cloudy solution.

25 mls F_1 and 10 mls of the protein solution were put in separate cellophane bags and dialysed for 20 hours against running water, to remove salts.

F_1 remained clear but its volume had increased to 46 mls. The protein solution after dialysis contained an insoluble precipitate of water insoluble proteins - euglobulins. The euglobulin fraction was filtered off to give filtrate F_2, 15 ml in volume. The precipitate was taken up in 6 ml 0.9% saline to give a cloudy solution R.

2 mls E, F_1, F_2 or R were then each incubated in duplicate with 2 mls Tyrode's solution + 2 mls M/5 Na_2HPO_4 + 1 ml histidine solution (15 mg/ml) for 3 hours at 38°C. After incubation, the histamine content of each mixture was determined.

The experiment was repeated as follows: 4.8gms of minced rabbit kidney was ground with silver sand and a mixture of 25.5 mls 0.9% saline + 12.5 mls M/5/

M/5 Na_2HPO_4 and then centrifuged twice, once at 2,500 ✕ g and then at approximately 35,000 ✕ g to give a clear tissue extract E.   15 mls of saturated ammonium sulphate solution were added to 15 mls of extract E, and shaken mechanically for 30 minutes. The precipitated proteins were centrifuged off. The supernatant was called F_1. The precipitated proteins which were found to weigh 2.6 gm, very wet weight, were taken up in 10 mls 0.9% saline.

23 mls of F_1 and 10 mls of the precipitated protein solution were dialysed for 20 hours. After dialysis, the volume of F_1 was found to have increased to 45 mls and was a clear solution. The precipitated protein solution's volume had increased to 15 mls, and contained a precipitate of water insoluble euglobulins which was filtered off. The filtrate was called F_2.   The euglobulin residue, which was found to weigh 0.5 gm wet weight was taken up in 10 mls 0.9% saline to give a cloudy solution R.

Incubation mixtures were made up in duplicate as follows: 4 mls E, 8 mls dialysed F_1, 4 mls F_2 or 5 mls R, were incubated with 2 mls Tyrode's solution+ 2 mls M/5 Na_2HPO_4 solution + 1 ml histidine solution. for 3 hours at 38°C.   To one mixture of each sample one/

one drop of benzene was added.

The results of these two experiments are given in Table 65.   The results for experiment 1 are the average of two incubations, but in experiment 2, the results are single results due to the large volumes of liquids which would be required for duplicate results.

TABLE 65/

TABLE 65

Histamine formed from histidine by the histidine decarboxylase present in a rabbit kidney extract and in the dialysed solutions prepared from the proteins precipitated by half saturating the rabbit kidney extract with ammonium sulphate.

SOLUTION	µg histamine found per incubation mixture.		µg histamine found per gm original tissue.	
	1st Expt.	2nd Expt.	1st Expt.	2nd Expt.
E Kidney Extract	1.9	5.4	2.5	10.8
" " + benzene	Not done	29.1	Not done	58.2
F_1 Dialysed supernatant after addition of $(NH_4)_2 SO_4$ soln.	Nil	Nil	Nil	Nil
" " + benzene	Not done	Nil	Not done	Nil
F_2 Water soluble proteins.	1.4	5.0	1.8	10.0
" " + benzene	Not done	23.0	Not done	46.0
R Saline soluble euglobulins	Nil	Nil	Nil	Nil
" " + benzene	Not done	Nil	Not done	Nil

The results showed conclusively that the enzyme histidine decarboxylase was precipitated with the proteins/

proteins by half saturation of the enzyme solution
with ammonium sulphate. After re-solution of the
precipitate in saline followed by exhaustive
dialysis, the enzyme was found in the water soluble
fraction of the proteins.

It was also found that the effect of benzene,
in increasing the yield of histamine from histidine,
prevailed, when the proteins were once more taken
into solution. This was final evidence that the
effect due to benzene, is an effect directly on the
enzyme in pure solution. Any cell bodies or
matter which persisted through the centrifugation
would have been removed in the euglobulin
precipitate.

This method, involving precipitation of the
enzyme by half saturation of its solution with
ammonium sulphate, followed by re-solution and
dialysis of the precipitated proteins, could not
be adapted to separating histidine decarboxylase
from histaminase, as the latter enzyme follows
the same path as histidine decarboxylase (Kapeller-
Adler, 1949).

DISCUSSION

The whole problem of the presence of an
enzyme in the mammalian body capable of decarboxy-
lating histidine with the formation of histamine and
its relation to the formation of histamine in the
body tissues, has been re-examined.

The present work supports in general the
results obtained by Werle and Holtz and their co-
workers, by demonstrating that there is present in
the kidneys of several rodents, a histidine decarb-
oxylating enzyme.   Such an enzyme has been detected
in the kidneys of rabbits, guinea-pigs, rats and
mice.

It was found that a tissue preparation con-
taining the enzyme, would produce, on incubation
with 15 mg histidine, a small quantity of histamine,
of the order of 1-20 µg histamine/gm tissue (Table
40), while under optimal conditions (i.e. in the
presence of benzene) the amount of histamine formed
was 6.0-114 µg histamine/gm tissue (Table 40).
Werle (1943) quotes the normal production of hista-
mine to be as high as 200 µg per incubation mixture
(probably 0.5 g tissue), but referring to his actual
work, this was found to be an extreme result from
several/

several hundred results and was obtained in the presence of toluene.   Generally, the figures quoted by Werle are in substantial agreement with those obtained in the present work, and which are shown in Table 40 (page 131).   The table shows some of the great variations in the histidine decarboxylase activities of the kidney tissue from several rabbits. The effect of toluene, in increasing the production of histamine, was never recognised by either Werle or Holtz.   In their experiments, toluene was added to the incubation mixtures on the assumption that it would be an effective bacteriostatic agent.   That it might have some other action was not considered.

The present results support the in vivo experiments carried out by Holtz and Credner (1944) and Schayer (1952b) in which an increase in the urinary excretion of histamine was noted after a subcutaneous injection of histidine, but not after an injection of histamine.   Schayer (1952b) further showed the presence of radioactive histamine in various tissues of the guinea-pig after a subcutaneous injection of radioactive histidine, again not found after an injection of radioactive histamine. Block and Pinoesch (1936) also claimed to have found an/

an increase in the histamine content of lung tissue after the subcutaneous injection of histidine.

It is considered that the results of the present work provide additional and conclusive evidence of the presence of an enzyme, histidine decarboxylase, in some tissues of rabbits, guinea-pigs, rats and mice.

The enzyme appears to be water soluble since it passes readily into a saline solution from minced tissue, consequently saline extracts can be utilised to study its distribution and properties.

Many tissues may contain so little enzyme or relatively large amounts of histaminase that the use of simple saline extracts would fail to show the presence of the enzyme and so possible methods of concentration of the enzyme and removal of histaminase were examined. Methods such as those used by Werle and Heitzer (1938) including precipitation of the enzyme from its solution by half saturation with ammonium sulphate, adsorption of the enzyme on aluminium hydroxide with subsequent elution or the removal of histaminase by adsorption on kaolin or celite, did not meet with success.

Though histidine decarboxylase is precipitated from its solution by half saturation with ammonium sulphate, it could not be separated from histaminase/

histaminase by this method, as the latter is also precipitated by the same procedure (Kapeller-Adler, 1949).

Although Werle and Heitzer (1938) claimed to have effected a 34-fold purification from rabbit kidney extracts, and some purification may have been obtained in the present experiments, the method was believed to be of no practical importance in trying to show the presence of small quantities of histidine decarboxylase in tissue extracts, and so these methods were abandoned in favour of aminoguanidine as a differential inhibitor to reduce the interference of histaminase, coupled with the use of benzene as a histidine decarboxylase potentiator.

The mammalian histidine decarboxylase was found to have a pH optimum of 8.3-8.6. Werle and Herrmann(1937) quoted the pH optimum as 9.0 while Holtz and Heise (1937b) found the pH optimum to be 8.0-8.2. However, Werle and his co-workers in most of their work actually used incubation pH's of 8.2-8.4.

In incubations of tissues and tissue extracts with histidine, the yield of histamine (μg histamine formed/g tissue/mg histidine) increased as the amount of histidine was decreased below 25 mg/g tissue, but the/

the absolute amount of histamine formed became so low
that in some experiments it might have been too low
to measure, thus 37.5 mg histidine/g tissue was used
in most experiments.

The best incubation time appeared to be 3-4
hours, though the maximum amounts of histamine were
formed in about 8-12 hours. About 80% of the total
histamine was formed in the first $3\frac{1}{2}$ hours, up to
which time the formation of histamine bore a linear
relation to the time. Between $3\frac{1}{2}$ and 12 hours incu-
bation the histamine formation falls off, ceasing
altogether after about 12 hours. The apparent
cessation of histamine formation may be due to
inhibition of the enzyme by histamine.

In much longer incubations it was observed
that after cessation of histamine formation at about
12 hours, there was a sudden and vigorous increase of
histamine production at about 24 hours. This was
believed to be due to bacterial action since this
phenomenon was not observed in incubation mixtures
which contained bacteriostatic agents such as chlor-
oform and benzene. However, if this was the explan-
ation, it demonstrated that bacteria were able to
form histamine at a pH much above the optimum and
outside the limits of the bacterial histidine
decarboxylase quoted (Gale, 1940; Epps, 1945).

It/

It was observed that the maximum histamine
production in the absence of benzene was never as
great as in the presence of benzene, and thus the
effect of benzene was not just to speed up the
reaction. When histamine formation restarted after
about 24 hours incubation in the absence of benzene
it increased far beyond the maximum obtained in the
presence of benzene.

As yet, no explanation has been found for the
results given in tables 25 and 26 which show that
less histamine was destroyed in the presence of l-
histidine than in its absence, when lung tissue was
incubated with histamine. It was believed that this
might be an indication of the power of the lung to
form histamine from histidine, since it had been
observed (Table 23) that l-histidine did not inhibit
the histaminase activity of a purified powder of
hog's kidney, demonstrated by Werle (1942). Accord-
ingly, it appeared that the histidine could affect
the destruction of histamine other than by inhibit-
ing histaminase. It might have been a true formation
of histamine. However, the results obtained were
small and sometimes non-existent.

Several experiments were designed to test if
lung tissue was capable of building up a store of
histamine in spite of the presence of histaminase
after/

after release of all the histamine it already con-
tained such as appeared _in vivo_. These experiments
included long incubation without histidine or
electrodialysis or the use of histamine liberators:
followed by the subsequent incubation of the tissue
with histidine.   The results of these experiments
were all negative.

No further evidence was forthcoming to shed
any light on either the structure or the action of
the enzyme, and so the theory put forward by Werle
and Koch (1949) must still remain, together with
their objections to the theories of Holtz (1941) and
Knoops (1938).   In considering some of the results
of experiments carried out by Werle and Koch (1949)
to support their theories of action of the enzyme,
it must be remembered that although the results of
an experiment, such as the one already described
on page 15 in which they obtained a 5-7% yield of
histamine when histidine and pyridoxal phosphate was
allowed to react, may be very small, they may still
be significant. This yield is much greater than the
formation of 25 μg histamine from 15 mg histidine
which resulted when a tissue extract containing the
enzyme was allowed to react with histidine.   This
represents only a yield of 0.2%.

In the search for a histaminase inhibitor
using/

using cat kidney tissue as the enzyme source, it was
expected that "Marsilid" (1-isonicotenoyl-2-isopropyl-
hydrazine) would be more active than aminoguanidine
for the following reason: Schayer (1953a, 1953b) as
a result of his experiments on the urinary excretion
products of histamine after administration of histi-
dine labelled with a C^{14} atom, believed that <u>in vivo</u>
there were two histamine destroying enzymes, hista-
minase and "histamine destroying enzyme II".   He con-
sidered that in the cat, the latter was the chief
histamine destroying enzyme, and that it was blocked
by "Marsilid", while histaminase was blocked by amino-
guanidine.   In the <u>in vitro</u> tests carried out in
the present work, it was found that, contrary to
expectations, "Marsilid" was of no value as a blocking
agent for the histamine destroying activity of cat
kidney tissue, being 1,000 times less active than
aminoguanidine.

In the histaminase-inhibitor studies, com-
parable results were obtained using fresh cat kidney
tissue or an acetone dried purified powder of hog
kidney (histaminase preparation of Kapeller-Adler)
as the enzyme source.

While studying the distribution of the enzyme
in the various tissues of selected animals, Holtz
and co-workers (Holtz, Heise, 1937b; Holtz, Heise
and/

and Reinhold, 1939; Holtz, Heise and Spreyer, 1938)
first shook the tissue extracts with kaolin to remove
any histaminase present. This was considered to be
unsatisfactory because kaolin can adsorb histidine
decarboxylase as well as histaminase. Werle and
Krautzun, (1938) took no such precaution and incubated
in the presence of histaminase if that was present.
Thus it was felt that the use of aminoguanidine to
block the histaminase activity was much more satis-
factory.   That it was effective is shown in Table
36 in which it will be seen that in most cases, even
in the presence of histaminase, as much histamine
was found in the incubation mixture containing amino-
guanidine but lacking in histidine decarboxylase
activity, as was found in the histamine blanks
given no incubation.

The use of benzene along with the amino-
guanidine enabled the optimum conditions for histi-
dine decarboxylase activity to be attained.   Werle
and Holtz and their co-workers were fortunate in
many of their experiments to have toluene present,
which has been shown to increase the activity of
histidine decarboxylase, but they did not recognise
its significance. They had toluene present in all
incubations of 8 hours or longer and in several of
shorter duration.   Long and short incubation
times/

times, in the presence and absence of toluene appear
to have been used indiscriminately by the authors.

Further criticism of these two authors seems
justified, particularly of Werle and co-workers,
because they only give single results and never
state whether they are the average of more than one
result or as they appear, single results. If
they are the latter, then some of the low results
reported cannot be considered significant. Further,
in many large experiments, all the tissue cannot
have come from one animal but separate control
values are not given. From Table 40, it is
apparent that control values obtained from one
animal's tissue cannot possibly be used for
another animals' due to the large variation in
the histidine decarboxylase content of the tissues
from different animals. Sometimes they have used
insufficient blanks to check particular effects,
thus in testing inhibitory or potentiating
substances, the substance was not added after
incubation. As a result, effects of these
substances on the guinea-pig ileum during assay
cannot be ruled out.

Moreover, in various experiments Werle et
al continually changed the incubation times
between/

between $1\frac{1}{2}$ and 24 hours and the incubation mixture volume between $1\frac{1}{2}$ and 10 mls. This latter variation itself altering the concentrations of the substrate and other added substances. Any of these alterations could change the results if compared with another incubation which was not identical. On the whole, in many experiments, insufficient details were given to justify the conclusions drawn from the results.

In the experiments by Holtz and his co-workers on the distribution of histidine decarboxylase activity the tissues were incubated with and without histidine, and after incubation the incubation mixtures were tested directly on the cat blood pressure preparation. Any difference in the fall of blood pressure produced on injecting equal volumes of the incubation mixture containing histidine and that lacking histidine were attributed to histamine formed by the tissue. But the compositions of these two solutions at the time of testing were not identical in respect of constituents other than histamine. Histidine should have been added to the incubation mixture lacking it, after incubation and directly before assay. An observed difference of the depressor activity of the two incubation mixtures could have been due either to a contamination of/

of the histidine sample with histamine, or a direct
effect on the blood pressure due to the histidine
itself. The possibility of errors due to these
causes is not discussed. In duplicate tests
carried out using neutralised histidine in 0.9%
saline, the present author has found that an inject-
ion of histidine solution containing an equivalent
amount of histidine in an equal volume to that used
by Holtz et al, produced a small fall in blood
pressure. On this theory of contamination of the
histidine by histamine, the negative results obtain-
ed in some instances by Holtz et al could be account-
ed for by destruction of the contaminating histamine
by the histaminase present. Holtz and co-workers
did no quantitative assays and so no idea of the
amounts of histamine formed can be obtained.

The enzyme histidine decarboxylase appears to
be generally distributed in the kidney, liver,
stomach and duodenum of the rabbit, guinea-pig,
mouse and rat, and also in the ileum of rabbits and
guinea-pigs. It appeared to be absent from the
other tissues tested such as the colon, caecum,
pancreas, spleen, lung, skin and sketetal muscle
of the animals already mentioned. It was absent
from/

from all these tissues of the cat and dog. In the stomach of the rabbit, the histidine decarboxylase was found in the inner mucosal and muscularis layers, but not in the outer layers, and there appeared to be more enzyme in the pyloric than in the cardiac portion.

In the rodents examined, histaminase and histamine appeared to be present together in the duodenum, ileum, lung and caecum, and to some extent in the colon. Of these tissues, only the duodenum of each animal and the ileum of rabbits and guinea-pigs contained any histidine decarboxylase. The stomach in every species contained large quantities of histamine and some histidine decarboxylase, but no histaminase.

In the cat and the dog no histidine decarboxylase was found anywhere in the tissues, while high histaminase activity, much greater than in any tissue of the rodents was found in the kidney, duodenum and ileum, being highest in the kidney. Comparatively smaller amounts were also found in the lung and pancreas.

The results for the distribution of histidine decarboxylase (Table 37) in the cat, dog, rabbit, guinea-pig, mouse and rat, differs in some respects. from those obtained by Werle (1943), (Table I). The most/

most important difference is that Werle quotes the
presence of histidine decarboxylase in the kidney
and liver of the cat.   This appears to be a cross
reference to the work of Holtz and Heise (1937b)
and Holtz, Heise and Spreyer (1938).   As already
discussed (page 207) these reported results may be
due to histamine contamination of the histidine
sample used or actually due to the histidine itself.
Also, for these reasons doubts are thrown on several
other results given in Table 1 for the histidine
decarboxylase activity reported in the liver of pig
and hen and the kidney of pig, hen, sheep and ox,
since these also appear to be based on the works of
Holtz and co-workers.

The rest of Table 1 is a review of some of
Werle's earlier work (Werle and Krautzun, 1938) in
which no experimental details or results were given
but merely a distribution table expressed in
arbitrary figures.   Werle (1940) claimed to have
found histidine decarboxylase activity in the small
intestine, caecum and colon of guinea-pigs and
rabbits, but the amounts are small and only single
results are cited.   Furthermore, Holtz, Credner and
Reinhold (1939) could find no evidence of histidine
decarboxylase activity in the large intestine or
rectum of the guinea-pig or in the intestine of cat,
dog,/

dog, sheep, ox, rat, mouse or goat. Besides this, Holtz and Heise (1937b) report that they could find no histidine decarboxylase in rabbit-lung, spleen, pancreas, skeletal and heart muscle, cat-lung spleen, pancreas and duodenum, or dog and calf pancreas.

Evidence for the cellular distribution of the enzyme was not obtained by differential centrifugation, for as it will be observed (Table 50) a tissue extract subjected to a gravitational force of 90,000$\times$g had an enzyme activity comparable to the original minced tissue. The centrifuged extract would of course, contain no particulate cell components. Thus the results indicate that the enzyme readily passes from the cells into the surrounding media, and so its presence in any particular fraction could not be shown. The same was found to be the case for histaminase.

However, it was shown that about 66% of the total histamine found in rabbit-lung tissue was present in the mitochondrial fraction. The remaining histamine was found in the supernatant after centrifugation at 90,000 $\times$ g and had probably been liberated post mortem. This agrees with the work of Copenhaver, Nagler and Goth (1953), and confirmed by Hagen/

Hagen (1954), who showed that the major part of the histamine in the dog liver was to be found in the mitochondrial fraction.

Price, Miller and Miller (1949) reported 28-45% of the total pyridoxal phosphate activity of rat and mouse livers was present in the mitochondria and 45-65% in the final supernatant, while only 4-8% was found in the microsomes. On the basis of this and other evidence, Schneider (1953) suggested that transaminases and decarboxylases may occur in the mitochondria.

It has still to be shown for certain if histamine is a local hormone and necessary to the body in the same way as acetylcholine and adrenaline. Although histamine produces many pharmacological actions on administration parenterally, it is still a matter of controversy whether any of these represents an intensification of a physiological response produced by the histamine normally occurring in the body.

Having shown the existence of a histidine decarboxylating enzyme, in some tissues of a few mammals, it must now be considered if the chief mode of formation of histamine, present in mammalian tissues, is the decarboxylation of exogenous histidine by/

by the enzyme histidine decarboxylase present in the tissue, or if the histamine present in the tissue originates from histamine absorbed from the intestine in which it is formed by bacterial action.

The enzyme histidine decarboxylase has only been demonstrated conclusively in a very few animals, all of which are rodents. The strongest evidence in support of the theory that this enzyme is present in mammalian tissues and that the histamine in the body is formed from exogenous histidine by it, is based entirely on evidence obtained from experiments performed on these rodents.

Thus the works of Werle and co-workers and Holtz and co-workers, have been performed on tissue pieces from rodents while the work of Holtz and Credner (1944) on the urinary excretion of histamine after injections of histidine and the final supporting evidence from the work of Schayer, 1952 in which he showed some radioactive histidine was converted into histamine in the tissues of guinea-pigs _in vivo_ after an injection of radioactive histidine, have both been carried out on guinea-pigs. In this animal, the presence of histidine decarboxylase has been conclusively demonstrated in several tissues, and it is therefore quite possible that systemic histidine is converted to histamine by these tissues. Thus all this/

this work does not prove that histamine is formed from histidine in the tissues of all mammals, but only in the rodents in which it has been investigated, particularly the guinea-pig.   Even so, it does not prove that this is the only source of histamine in these herbivorous animals as it may be secondary to some other source common to all mammals.

Wilson (1954) demonstrated that an interference with the fauna and flora of the intestine of rats, such as a reduction in their number by the use of orally administered antibiotics or sulphonamides there is a decrease in the urinary excretion of histamine compared with control animals.   These experiments seem to support an alternative theory to that already mentioned, namely, that the histamine occurring in the mammalian tissues originates from histamine absorbed from the intestine, where it is formed as a result of bacterial activity.

The chief evidence clai med against this theory, depends on the presence of histaminase in the intestine wall, which is supposedly present to prevent the entry of histamine. This evidence is not necessarily correct. Some of the histamine formed in the intestine by the bacteria may be combined to protein or some other molecule (Rocha e Silva, 1943; Rocha e Silva and Andrada, 1943) and may be absorbed as/

as such a complex compound, in a pharmacologically inactive but easily convertible form, which is not attacked by histaminase. The histaminase could be present to prevent the entry into the body of any free pharmacologically active histamine. Alternatively, the amount of histamine to be absorbed may be greater than that which can be destroyed by the histaminase in the intestine wall, and so a certain amount enters the body.

The chief evidence obtained from the present work in support of the theory that histamine in the body tissues is absorbed from the intestine, is from the fact that in both the cat and the dog, the presence of histidine decarboxylase has not been demonstrated, as would be expected if histamine was formed from histidine in the tissue by such an enzyme. On the contrary, much larger amounts of histaminase were found in cat and dog tissues, suggesting the presence of larger amounts of histamine.

Thus sufficient histamine may be absorbed from the gut in the case of carnivorous animals to be available for all possible physiological necessities, so that no endogenous formation of histamine is necessary and consequently there is no need for the presence of a histamine forming enzyme. On the other/

other hand, in the case of the herbivorous rodents
it seems possible that insufficient histamine may be
absorbed from exogenous sources and to make good the
deficit, histamine must be formed from histidine in
certain tissues of these animals by the action of an
enzyme, histidine decarboxylase, which is found in
the kidney, liver and duodenum.   There may be some
relation between the distribution of the enzymes
histidine decarboxylase and histaminase and the diet
of animals. The effect of diet may be secondary to
the presence of different fauna and flora in the gut.

It would be interesting to know what results
would be obtained if experiments, similar to those
performed by Schayer (1952) and Holtz and Credner
(1944), were performed on cats or dogs instead of
guinea-pigs, since the presence of histidine decarb-
oxylase has not been demonstrated in these animals,
and much greater quantities of histaminase are pre-
sent in the tissues. Further, it would be interest-
ing to see if results similar to those obtained
using rats, would be found if Wilson's work (1954)
was also repeated on cats or dogs, and again on
guinea-pigs.

Thus in conclusion, though proof of the
presence of a histidine decarboxylating enzyme in the
kidneys, livers and duodenal tissues of certain
specified/

specified rodents has been presented, it does not appear that this is the major pathway in the formation of histamine in carnivorous animals and indeed it may only be a secondary route for the formation of histamine in the herbivorous animals described.

The effect of benzene and other organic solvents.

During this work, an interesting observation was made. During the course of incubations longer than 8 hours duration, chloroform (22 mg) was added to the incubation mixtures as a bacteriostatic agent. When the effect of this quantity of chloroform on a shorter incubation was tested, it was found that the chloroform itself apparently increased the production of histamine 3-4-fold. By various tests, it was demonstrated that this was a direct potentiation of histamine formation and not an artefact effect due to any chloroform remaining, after the incubation mixture was boiled at the end of the incubation, on the guinea-pig ileum during assay, nor was the increased histamine yield due to a release of histamine from the tissues. That the gut active substance formed in the presence of chloroform was histamine or a histamine-like substance was shown by the use of mepyramine (Reuse, 1947).

As a result of this observation, a series of organic/

organic solvents was tried. It was found that this potentiation was common, but in a varying degree, to a large number of organic solvents, both aliphatic and aromatic, of which the aromatic were the more potent. The only relation between the structure of the solvent and its potentiating activity noticed, was that the six membered benzene ring or pyridene ring were the most powerful. Any side chain substitution caused a decrease in the potentiating activity. Thus benzene, which appeared to have the greatest activity and did not inhibit in high doses, was used for further investigation.

So far as the present author is aware, the only mention, in the literature, of an enzyme being potentiated by an organic solvent, concerns the choline acetylase of brain tissue, but in all cases the effect was obtained only when the medium was the pure solvent. Stedman and Stedman (1939) reported that ether and chloroform promoted the formation of acetylcholine by ox and rat brain. This was confirmed by Mann, Tennenbaum and Quastel (1939) and later by Feldberg (1945).

The mode of action of benzene and the other organic solvents in the present experiments appears to be very different from the above reported examples. The effect is apparent in aqueous extracts of tissues and/

and when only minute amounts of the organic solvent are present.

That the effect of benzene and probably the other organic solvents was probably not due to a bacteriostatic and bactericidal action was shown by incubations in which first aureomycin and then sulphathiazole was incubated with rabbit kidney tissue without any potentiating action on the production of histamine.

Since a potentiating action was also shown by chloroform and benzene in concentrations at which they were totally soluble in the tissue extracts and was also shown by pyridine which is miscible with water in all proportions, it would appear to be unlikely that the potentiation of histamine formation by the organic solvents was due to any phase effects, due to concentration of the histamine in the solvent layer, so allowing the formation of more histamine. The conclusion appeared to be supported by the finding of no histamine in the benzene layer, when a tissue extract containing histamine was shaken with benzene.

A further possibility was that the potentiation was due to an effect exerted by the solvent on the cell membrane or the membrane of some cell constituent/

constituent, in that the solvent might have allowed
an easier passage across the membrane either for the
substrate to enter the cell or for the enzyme to
pass into the substrate medium, in either case
allowing better enzyme-substrate mixing.   However,
this seems unlikely, since a rabbit kidney tissue
extract, subjected to centrifugation at 90,000 $\times$ g
for one hour, still shows an increased production of
histamine from histidine when incubated in the
presence of benzene.   After centrifugation at this
force, all cell fragments contained in the original
tissue extract would have been precipitated (Schnie-
der, 1953) leaving only substances in colloidol form
or in pure solution.   Thus the effect of benzene
must be exerted on the enzyme, when the latter is
in solution.

Any explanation of this effect involving dis-
solution of lipids to which the enzyme might be
bound, seems to be improbable, because of the very
small quantities of organic solvent necessary to
produce the effect.   Further, if the enzyme was
attached to lipid, it would probably have been con-
tained in the small lipid layer at the surface of the
supernatant, after centrifugation at 90,000 $\times$ g.
This layer was tested, along with some of the aqueous
layer/

layer, and gave results almost equal to those for the aqueous layer.

The explanation of the action of benzene to increase the decarboxylation of histidine by the enzyme histidine decarboxylase may lie in the physical state of the enzyme in solution. It may be suggested that the enzyme molecules are formed into micelles. A micelle was defined by Posternak (1901) as "the smallest quantity of a colloid, possessing the physical properties of the colloid as a whole, and formed by the association of molecules of large size". Micelles are said to possess "adhesiveness", a property based on Duclaux's "molecular adhesion" theory. Micelles are commonly associated with the soaps, but are also associated with proteins, particularly the albumins.

On this supposition, the enzyme molecules when in solution may be clumped together. If this is so, only the active prosthetic groups of those enzyme molecules situated at the surface of the aggregate will be free to react with the substrate, while many more active groups will be buried inside the micelle and unable to act. It is tentatively suggested that the effect of benzene is to break up, partially or wholly, these micelles, as a result, many more prosthetic groups are freed and can then take/

take part in a reaction with the substrate, and so
more histidine is decarboxylated with the formation
of histamine. It appears that some organic molecular
structures are more active than others, in breaking
up the micelles and on the evidence of this work, the
unsubstituted benzene ring, or pyridine ring appear
to be the most active. We may observe two facts
about the benzene ring, either or both of which may
account for the greater activity. Firstly, the
benzene ring is a flat structure and may be able to
penetrate the micelle structure better, secondly,
the benzene ring is non-polar.

Some evidence for this theory might be
obtained by ultra high speed differential centri-
fugation of a tissue extract containing the enzyme
and another part of the same extract to which benzene
has been added. Centrifugation would be continued
until the enzyme was precipitated. If it was found
that the enzyme in the tissue extract was precipitated
at a speed lower than that required to precipitate
the enzyme in the extract containing benzene, this
would be good evidence that the benzene was breaking
down the micelles. Since the micelles are composed
of groups of molecules they might be expected to have
a faster sedimentation rate and so would be precipi-
tated first. If it was found that the precipitation
speeds were the same, this would not disprove the
theory/

theory, for the benzene may only open up the micelles or even split up relatively few of the micelles, in either case, more prosthetic groups would become available, and so there would be an increased production of histamine.

SUMMARY

1.   The problem of the formation of histamine
in the mammalian body, from histidine absorbed
from the gut, by a tissue enzyme histidine
decarboxylase, has been reinvestigated. There
was already evidence that some tissues con-
tained an enzyme capable of decarboxylating
histidine, with the formation of histamine.
The effects of pH, substrate concentration,
time of incubation and other factors
influencing the activity of this enzyme were
investigated, using rabbit kidney tissue as a
source of the enzyme. From these experiments,
a standardised technique was developed.

2.   It was then decided to try to increase
the production of histamine by the use of
chemical agents. This was however, unsuccess-
ful, until it was observed in a control experi-
ment that chloroform, added to the incubation
mixtures to inhibit bacterial growth during
long incubations had a very surprising effect
since/

since very small quantities increased the
production of histamine. This led to further
experiments with other organic solvents, many
of which were found to be active. Of these
benzene proved to be the most active,
increasing the yield of histamine 6-10-fold.

3.   As there was evidence that some tissues
contained both histidine decarboxylase and
histaminase, (a histamine destroying enzyme)
it seemed likely that the histaminase would
interfere with the estimation of the histidine
decarboxylase. It was therefore decided to
try to find a differential inhibitor of
histaminase, and then reinvestigate the
distribution of histidine decarboxylase,
using this inhibitor, together with the
presence of benzene in the incubation mixture.

4.   Wet cat kidney tissue was found to be a
potent source of histaminase, when incubated
in Tyrode's solution buffered at pH 7.2. Thus
many chemical substances, chosen from the
literature, were incubated at various concen-
trations with rabbit kidney tissue or cat
kidney/

kidney tissue together with the appropriate
substrate, and the inhibition, caused by the
substance, of histidine decarboxylase and of
histaminase respectively, noted. Amino-
guanidine was found to be a specific inhibitor
of histaminase. Thus the distribution of
histidine decarboxylase was restudied in the
presence of 10^{-4} aminoguanidine and $2x10^{-3}$
benzene.

5.   The distribution of histidine decarboxy-
lase in the tissues of several laboratory
animals showed that the enzyme was present in
the kidney, liver, stomach, and the upper part
of the small intestine of the common rodents,
such as the rabbit, guinea-pig, rat and mouse.
It was not detected in the caecum, colon, lung,
skin, voluntary muscle or spleen of these
animals. There was no evidence of the presence
of this enzyme in any of the tissues of dogs or
cats.

6.   The distribution of histaminase was
also studied in the tissues of the same
animals, and in contrast to the distribution
of/

of histidine decarboxylase, the richest
sources of the enzyme were the kidneys of
dogs and cats, while the enzyme was completely
absent from the kidneys of rodents.
Histaminase was present in the alimentary
tract of all the animals studied, but was
generally located lower down the canal than
histidine decarboxylase, being absent in the
stomach, but present in the colon, with the
maximum concentration occurring in the ileum
rather than the duodenum. Histaminase was
absent from caecum, skin, stomach and voluntary
muscle, and was sometimes found in the lungs,
but in a low concentration.

7.   The distribution of histidine decarboxy-
lase, histaminase and histamine in the cell
constituents was studied, by differential high
speed centrifugation. It was concluded that
the histidine decarboxylase of rabbit kidney
tissue and the histaminase of rabbit lung
tissue was probably in the cytoplasm while the
histamine was probably in the mitochondria.

8.   The possible significance of histidine
decarboxylase/

decarboxylase as a secondary pathway in the
formation of histamine is discussed.

9.   A separate set of experiments were
performed in an endeavour to discover the
mechanism of the potentiation of histamine formation
shown by benzene and the other organic solvents.
The possibility that the effect is produced by
the action of the organic solvents on the
molescules of the enzyme in pure solution, has
been discussed.

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