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T H E S I S

for the Degree of Ph.D. 1922

by

CHARLES ROBERT HARINGTON.

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AN ASPECT of the PATHOLOGY of PROTEIN METABOLISM.



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AN ASPECT of the PATHOLOGY of PROTEIN METABOLISM.

Introduction -

Various clinical conditions are commonly grouped under the heading of "intestinal intoxication".

There are two distinct sets of symptoms which appear to be attributable to toxæmias of intestinal origin, the first being characterised by general depression and headaches accompanied by a reduced blood pressure, and the second associated with lowered sugar tolerance and a high blood pressure, showing, in fact what appears to be a general stimulation of the sympathetic nervous system.(1)

If these symptoms are due to the absorption of a toxin from the intestine, the reason for their manifestation must be sought in the abnormal length of time during which the intestinal contents are retained, and it is necessary therefore to consider carefully the possibilities raised by this abnormal retention.

In the first place there is no evidence that /

that any of the normal end products of digestion would produce toxic effects even if absorbed in abnormally large quantities. Undigested proteins might conceivably be toxic but are unlikely to be absorbed; moreover the symptoms observed do not correspond to those produced, presumably by the passage into the blood stream of minute quantities of unsplit proteins, (2) in specially sensitive individuals. It does not therefore seem possible to account for the phenomena of intestinal intoxication on the theory of absorption of undigested or semi-digested protein, and we are driven to consider other possibilities.

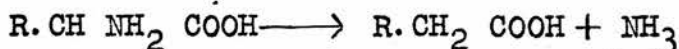
The prolonged retention of the intestinal contents would obviously favour bacterial activity and the question presents itself as to whether the solution of the problem may be found in this direction. Setting aside the case of infection with a specific organism which is not normally present in the intestine, such as the bacillus of typhoid, cholera etc., we are left with two possibilities; one or more of the normally occurring bacteria may flourish to an abnormal extent, increasing in numbers or virulence or both, and, by its own endogenous toxin, may produce the symptoms of disease, or the toxin /

toxin responsible for these symptoms may be produced as an exogenous metabolic product of one or more organisms in the course of the action of the latter on the digestion products of the food.

In herbivora undoubtedly, and probably to some extent also in carnivora and omnivora, bacterial action plays a part in the normal course of digestion, in particular in the digestion of cellulose; but as the result of this action in carbohydrate metabolism no harmful substances are known to be produced, nor does it seem likely that they would be so under any circumstances.

If we consider, however, the products of protein digestion in relation to bacterial action, we see a different picture. By the time that the food has reached the large intestine where the bacterial action occurs, the proteins will, for the most part, have been hydrolysed to their constituent amino-acids, and it is the latter which we must regard as possible precursors of toxic substances.

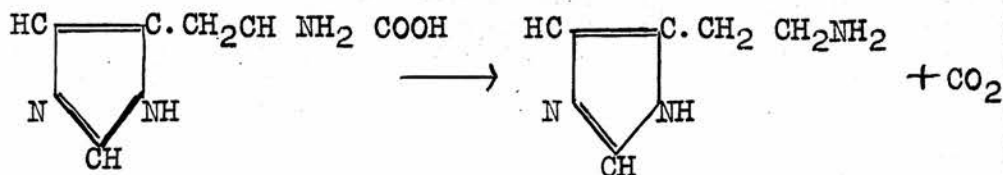
Now an amino-acid, when subjected to the action of bacteria undergoes usually one of two changes; it either loses its amino-group, being converted to the corresponding fatty acid



or it loses carbon dioxide, being converted into an amine : $R.CH NH_2 COOH \longrightarrow R.CH_2 NH_2 + CO_2$.

The fatty acids and their further oxidation products such as the keto- and hydroxy-acids, are physiologically inactive substances and, if present in the intestine, would produce no ill-effects; the amines, however, are in some cases exceedingly toxic.

The most active physiologically of the amines formed by the decarboxylation of the known amino-acids is 4 (or 5) β iminazolyl ethylamine (histamine) formed from histidine by the loss of carbon dioxide :

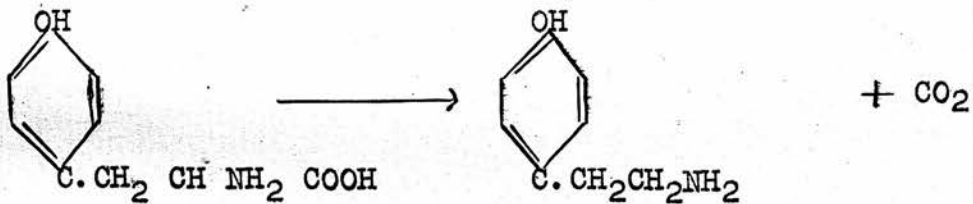


This particular reaction is accomplished by certain bacteria with great ease, so much so that a patented commercial process for the preparation of histamine depends on the use of a certain organism.

The principal physiological action of histamine is as a stimulant to all plain muscle, in particular that of the uterus; it is principally to histamine that ergot owes its ebolic effect; his-
tamine /

histamine however differs from other related amines in causing a fall of blood pressure; this effect is not constant to all species but occurs in man and the carnivora; it is due to the action of the amine as a capillary poison which is a property peculiar to itself; in common with the other amines of its class it constricts the arteries.

A typical member of this class is para-hydroxy phenylethylamine (tyramine) which is formed in a similar way from tyrosine



this substance produces a purely vaso-constrictor effect. Histamine, when injected intravenously, produces a general condition which closely resembles that of anaphylactic shock.

Now in view of the toxic effects which are known to be exerted by histamine it does not seem unreasonable to associate the symptoms observed in the first of the two mentioned classes of intestinal intoxication with this substance, since they are such as might conceivably be produced by the continuous absorption /

absorption of small amounts of it, and since the conditions prevailing in the intestine are such as would facilitate its production as the result of the action of bacteria upon the histidine derived from the food.

A certain amount of evidence has been brought forward by Graham Brown (1) which seems to relate the second (high blood pressure) type of intoxication to the the production in and absorption from the intestine of tyramine.

The investigation described below is an attempt to bring forward definite experimental evidence bearing upon the supposed relation of histamine to the first type of intestinal intoxication, and the principal questions dealt with are as follows :-

- (1) Is histamine present in the human intestine, and if so, under what conditions?
- (2) Can histamine be absorbed from any part of the normal intestine, and if so from what region is absorption most rapid?
- (3) Is the absorption of histamine from the intestine affected by damage to the intestinal mucosa?

(4) /

- (4) Supposing that histamine is absorbed, is it subsequently destroyed in the liver, so as to be innocuous to the organism?
-

PART I.

The Presence of Histamine in the Human Intestine.

The bacterial action which occurs in the intestine takes place to the greatest extent in the caecum and the ascending and transverse colon, and it was therefore in intestinal contents from these regions that the presence of histamine was sought for.

The presence of histamine in the intestinal mucosa of the bullock has been demonstrated by Barger and Dale (3), and Mutch (4) has obtained from the human ileum an organism (unidentified) which is capable under aerobic conditions, of de-carboxylating histidine. The actual presence of histamine in the human /

human intestine has however never hitherto been proved, and it was resolved to attempt to isolate histamine from intestinal contents, or, if this should prove impossible, to demonstrate its presence therein by means of the sensitive reaction which is obtained with this substance in contact with the isolated uterus of the virgin guinea-pig. This physiological reaction, though not in itself at all specific for histamine, may be rendered practically so by suitable preliminary chemical treatment of the material, and it is possible by this means to demonstrate with comparative certainty the presence of amounts of histamine which are far too small to permit of chemical isolation.

In no case investigated was it found possible to obtain enough material for the chemical isolation of histamine, but in several cases, after careful preliminary treatment to eliminate other substances, a positive physiological reaction was given, and in these cases the presence of histamine may be regarded as established.

Experimental Part.

The material investigated consisted of
caecal /

caecal contents (cases 1, 2, 3, 6), contents of the transverse colon (cases 4 and 5) and faeces (cases 7 and 8). The caecal and colonic contents were obtained either by washing out the caecum (or colon) or were collected on dressings.

On collection they were treated as follows :-

In the case of washings, these were treated at once with mercuric chloride and hydrochloric acid to make a concentration in the whole of 0.5% of the former and 0.9% of the latter; when the material was obtained on dressings, the latter were placed at once on removal in a solution containing mercuric chloride and hydrochloric acid in the above-mentioned concentrations; in dealing with faeces (formed stools) these were thoroughly broken up and mixed with water, mercuric chloride and hydrochloric acid being added as before. In this manner it was possible to collect quantities of material which were fixed, as regards bacterial action, at the stage which they had reached in the intestine.

When a considerable quantity (1500 to 2500 c.c.) of material had been collected in this way, the whole mixture was boiled and filtered; this filtration was troublesome, and was almost impracticable without /

without previous boiling.

For the further working up of the material the first method tried was to make the solution alkaline, in order that the bases present might be precipitated by the excess of mercuric chloride; this however was not found to be satisfactory, since the mercuric chloride precipitated other interfering substances and did not completely precipitate the bases.

As the result of numerous experiments the following was found to be the most satisfactory procedure. After the first filtration, the solution which was clear, but in most cases highly coloured, was freed from excess of mercury by means of hydrogen sulphide; the mercuric sulphide was filtered off and the filtrate treated with lead acetate (first a solution of neutral lead acetate and then of the basic salt). This produced a bulky precipitate which was filtered off and well washed with water. The solution was then freed from excess of lead by means of hydrogen sulphide and the lead sulphide was filtered off.

At this stage the solution was almost colourless or light yellow. It was now concentrated on /

on the water bath in vacuo, the temperature being kept below 60° C., to a small volume; sulphuric acid was added to make a concentration of 5% and the solution was treated with phosphotungstic acid until there was no further precipitate; after standing overnight the precipitate was separated at the centrifuge, washed, dissolved in dilute acetone and decomposed with baryta. The filtrate from the barium phosphotungstate after completely freeing from barium with sulphuric acid was concentrated in vacuo, most of the acetone being removed by this means. The solution thus obtained was then fractionated with silver and baryta by the method of Kossel and Kutscher.

The precipitate obtained in the histidine fraction was separated at the centrifuge, well washed with water, suspended in distilled water and decomposed with hydrogen sulphide; the silver sulphide was filtered off and washed and the united filtrate and washings were thoroughly freed from excess of hydrogen sulphide by boiling under reduced pressure.

In cases 1 - 6 the solutions obtained on recovery from the silver precipitate gave Pauly's reaction with sodium diazo-benzene sulphonate, but with /

with considerable variations in intensity. In cases 7 and 8 no diazo reaction could be obtained.

The solutions at this stage were tested for physiological activity as follows :- A horn of the uterus of a virgin guinea-pig was suspended in a bath of oxygenated Ringer-Locke solution at 37°C. The sensitiveness of the uterus to histamine was first determined by finding the amount of the latter which it was necessary to add to the bath in order just to produce a contraction; the process was then repeated, substituting the solution to be tested for the histamine. In this way it was possible to form an approximate idea of the concentration of histamine present.

In order to eliminate the possibility of confusion between histamine and the other substances of unknown constitution which give a similar reaction with the uterus and which might conceivably be present, part of the solution, in two typical cases, (1 and 6) was heated in the boiling water bath for ten minutes with a concentration of 4% sodium hydroxide; the physiological test was repeated on this solution after cooling and neutralisation. In case 1 a certain diminution in activity was observed but in case 6 no such diminution could be found. There was therefore /

therefore present some physiologically active substance which, having regard to its method of isolation and also to the fact of its resistance to alkaline hydrolysis, must be presumed to be histamine. The variations in physiological activity in the different cases corresponded closely with the variations in the intensity of the diazo reaction.

Case Reports and Tables.

Case 1. M.M. Female. Age 40.

This patient had suffered for many months with irregular fever and intermittent distension of the abdomen. An exploratory operation on 29/7/20 was performed and nothing abnormal was found. She was admitted to the Royal Infirmary on 16/9/20 and while under observation symptoms of acute intestinal obstruction developed. Mr. Graham operated on 22/9/20 and although the whole of the caecum and colon were found greatly distended, no definite point of obstruction was evident. A caecostomy opening was made which continued to function satisfactorily and between December 24th and 29th 1920 the material discharged from the caecostomy was collected and examined for histamine. On further examination there was /

was found evidence of obstruction at the splenic flexure. A second operation was performed by Mr. Jardine on 19/3/21 when adhesions and kinking of the splenic flexure were found producing acute intermittent obstruction. Anastomosis was performed at this site and the patient made an uninterrupted recovery.

Case 2. Mrs. I. Age 54.

This patient gave a history of intermittent diarrhoea without constipation covering a period of one year. On 10/1/21 she developed signs of acute intestinal obstruction. At operation on 15/1/21 Mr. Graham found great distension of the small intestines, caecum, transverse, descending and upper part of pelvic colon. In the lower part of pelvic colon a malignant stricture was found. Caecostomy was performed which continued to function satisfactorily for some months when the patient returned to have the malignant growth removed. On 5/2/21 the contents discharging from the caecostomy opening were collected and examined for the presence of histamine.

Case 3. /

Case 3. A.T. Male. Age 59.

For some years this patient had been troubled with dyspepsia but early in February 1921 he became acutely ill with severe pain in the lower abdomen and vomiting, followed by obstinate constipation in spite of purgatives. Enemas however gave satisfactory results, relieving the vomiting. On 7/3/21 an operation was performed by Sir Harold Stiles and there was found a malignant stricture at the junction of the iliac and pelvic colon while the caecum, transverse colon and descending colon were much distended. Caecostomy was performed which continued to function satisfactorily. On 1/4/21 and again on 10/4/21 the contents from the caecostomy were collected and examined for histamine.

Case 4. G.L.K. Age 67.

For several months the patient had complained of obstinate constipation with pain in the lower abdomen which was paroxysmal in character and frequently accompanied by vomiting. On 20/2/21 symptoms of complete obstruction developed and he was admitted to the Royal Infirmary on 1/3/21 for intestinal obstruction. At operation Sir James Hodsdon found a malignant growth with many adhesions involving /

involving the gall-bladder, liver and transverse colon. A colostomy opening was made about the middle of the transverse colon which continued satisfactorily. On 4/4/21 material from colostomy was collected and examined for histamine.

Case 5. Mrs. J.G. Age 62.

For several months patient had complained of epigastric pain, vomiting and constipation. On 15/2/21 these symptoms became very extreme and signs of acute intestinal obstruction developed. Patient was admitted on this date and Sir James Hodsdon operated immediately, when a carcinoma of the upper part of the pelvic colon was found. A preliminary colostomy opening was made in the transverse colon which continued to function satisfactorily. On 1/4/21 material from the colostomy was collected and examined for histamine.

Case 6. T.E. Male. Age 48.

In September 1919 patient began to suffer from pain across the lower abdomen with flatulence and gaseous eructations. He gradually became more and more constipated. Suddenly in October of that year the constipation became complete and he had great distension /

distension of the abdomen, followed by vomiting. He was admitted to the Royal Infirmary on October 13th 1919 when he was operated on by Mr. Wilkie for carcinoma of the pelvic colon. At operation this diagnosis was confirmed, a large tumour was removed and colostomy was performed. On December 23rd patient returned to the Infirmary and was again operated upon by Mr. Wilkie and the colostomy closed. He continued in good health for about a year when he was readmitted to the Infirmary on 31/3/21, suffering from recurring attacks of pain in the abdomen, which was relieved by the passage of flatus. On examination the abdomen was distended, very tympanitic, with tenderness and rigidity in the right iliac fossa. A tumour was palpable which was hard, irregular and adherent to the scar of the previous operation. There was pronounced constipation and recurrent attacks of vomiting. On 1/4/21 patient was operated on by Mr. Wilkie. On opening the abdomen a considerably quantity of yellowish fluid escaped and numerous nodules were found in the peritoneum and in the omentum, which were found to be carcinomatous, and there was a large mass found in the right iliac fossa. The caecum was brought to the surface and caecostomy performed. This continued to function very well and on 16/4/21 the contents from the caecostomy were collected /

collected and examined for histamine.

Case 7. K. McK. Male. Age 32.

For 15 years this patient had been troubled with headaches and "biliousness" with increasing mental depression. In 1917 his appendix was removed and some months later he was operated upon for intestinal obstruction when 3 feet of the ileum was resected. After these operations he suffered from diarrhoea. In Spring of 1920 he was again operated on for the separation of adhesions, after which he had temporary relief. In September 1920 another operation was performed by Mr. Wilkie when it was deemed advisable to do a short-circuiting and the terminal portion of the ileum was anastomosed to the upper part of the ascending colon. His condition did not improve and he continued to suffer from sleeplessness, headache and profound nervous depression. A barium meal demonstrated the caecum to be large and flaccid and apparently in a very atonic condition. It would fill with ease up to the level of the ileo-colonic opening when there would be distinct syphonage and regurgitation into the ileum. A barium enema demonstrated this regurgitation in a most pronounced degree. The stools were collected from the 15th to /

to 20/1/21 and examined for histamine.

Case 8. M.B. Female. Age 10.

This patient suffered from chronic nephritis
without any evidence of intestinal disturbance.

She was on milk diet. On 13th to 15/1/21

her stools were collected and examined for
histamine.

Description /

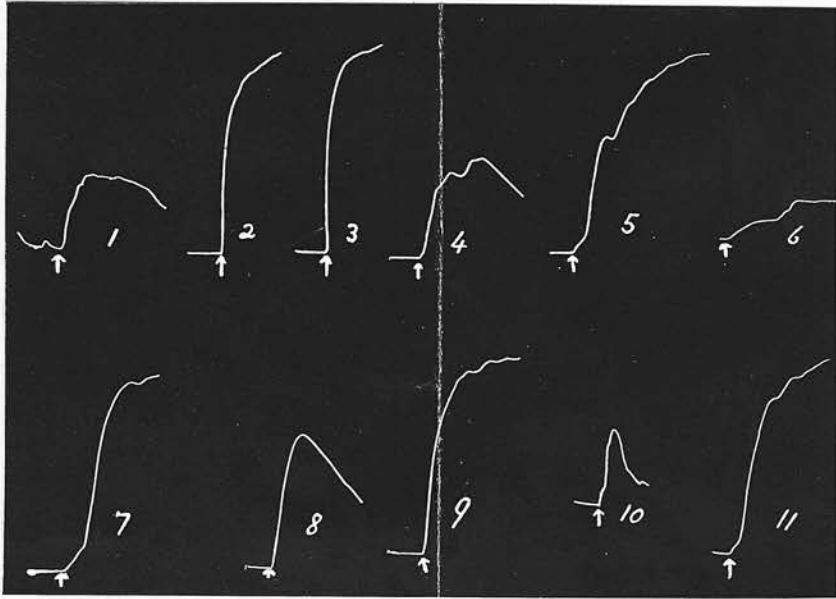


Plate Ia.

DESCRIPTION of PLATE Ia.

1.	Histamine solution 1 : 16.6 millions		= submaximal contraction.
2.	Histamine solution 1 : 12.5 "		= maximal contraction.
3.	Histamine (hydrolysed) solution 1 : 12.5 millions		= maximal contraction.
4.	Case No. 1 Non hydrolysed fraction. Dilution 1 : 85		= submaximal contraction.
5.	" " " " Dilution 1 : 72		= maximal contraction.
6.	" " Hydrolysed fraction. Dilution 1 : 50		= submaximal contraction.
7.	" " " " Dilution 1 : 37		= maximal contraction.
8.	Case No. 6. Non hydrolysed fraction. Dilution 1 : 335		= submaximal contraction.
9.	" " " " Dilution 1 : 250		= maximal contraction.
10.	" " Hydrolysed fraction Dilution 1 : 278		= submaximal contraction.
11.	" " " " Dilution 1 : 250		= maximal contraction.

The presence of histamine was demonstrated in cases 1 - 6 but not in cases 7 and 8, i.e. in the contents of the caecum and transverse colon, but not in the faeces. The largest amount was obtained in case 2, where the activity of the solution, by comparison with one of pure histamine, corresponded to a concentration of 1:10,000 of the latter.

The complete record of the physiological test in the typical cases 1 and 6 is shown in Plate Ia and the other cases are recorded in Table Ia. Owing to the uncertainty of the amount of dilution of the original material, and the far from quantitative nature of the method of working up, it is impossible to form an accurate estimate of the concentration in which histamine was present in the intestine. In case 1, from a consideration of the various factors, 1:100,000 was arrived at as being a figure expressing the order of magnitude of the original concentration.

From the results of the experiments described and from a consideration of the notes on the cases, it appears that histamine does occur in small amounts in the human intestine, but that this occurrence is not dependent upon the existence of a retention /

retention of intestinal contents, since it is found a considerable period after the operative relief of obstruction. The absence of histamine from the faeces is probably accounted for by the oxidation of this substance during its passage through the large bowel.

? why oxidation?
Why not absorption?

PART II.

The Absorption of Histamine from the Normal Intestine.

Having thus shown that histamine is without doubt occasionally, and probably usually present in the human intestine, the next problem to be considered was the question of its absorption.

Mellanby (5), in the course of numerous experiments upon the absorption of histamine from the intestine of cats, found that the rate of disappearance of histamine from the intestine increases on passing down the small intestine towards the caecum. That the disappearance of histamine may be /

be accounted for by absorption, was indicated in certain experiments by a profound fall of blood pressure when this was recorded. He also states that he found no evidence of disappearance from the caecum.

The experiments about to be described confirm the results of Mellanby with regard to the relatively high rate of absorption from the ileum; but in addition distinct evidence has been obtained of its absorption from the caecum.

Experimental Part

The experiments were carried out as follows :-
Cats were always used under paraldehyde anaesthesia, a little ether being also used early in the experiment. Tracheotomy was first performed; a cannula for the blood pressure record was inserted in the carotid artery and then the abdomen was opened and the part of the intestine to be experimented on was isolated and tied off. A cannula was inserted into the loop of intestine for the injection of the solution of histamine; this eliminated the possibility of the direct introduction of a trace of histamine into /

into the circulation, such as might occur during the piercing of the intestinal wall with the needle of the syringe. In the case of the experiments on the caecum, the cannula was introduced through the ileo-caecal valve, and the caecum ligated about it, so as to prevent the possibility of regurgitation into the ileum. In the case of female cats a tracing of the uterine contractions was taken in addition to tracings of respiration and blood pressure; in these experiments the animal was immersed bodily in a bath of saline at 36° - 37° C. and the movements of the uterus were recorded by means of the Cushny myocardiograph.

The histamine used was a solution of the acid phosphate in normal saline, containing 1% of histamine. This solution was warmed to 37° C. before injection, the syringe and cannula being washed out with a few c.c. of warm saline.

In Table I and figs. 1 to 5 will be found the results of these experiments.

The following points call for special attention. In the first place the fall of blood pressure per minute is most rapid after injection into the ileum, slightly less so after introduction into the duodenum, and very slow after the introduction of similar and larger doses into the stomach and caecum. In the majority of cases the introduction of the histamine is followed after a latent period of a few seconds by a sharp fall of blood pressure which is usually complete at the end of 15 to 25 seconds. This fall may be followed by a certain amount of recovery. As this delay or recovery passes off there then ensues a slow but steady fall of blood pressure. These three phases correspond to those observed by Dale and Laidlaw (6) as the result of intravenous injections of histamine. The first two correspond closely in point of time, duration and degree to what these authors found, except that they are slightly delayed. The third phase is much more gradual than after intravenous injection. In the case of the caecum, recovery from the primary fall may be almost complete and the /

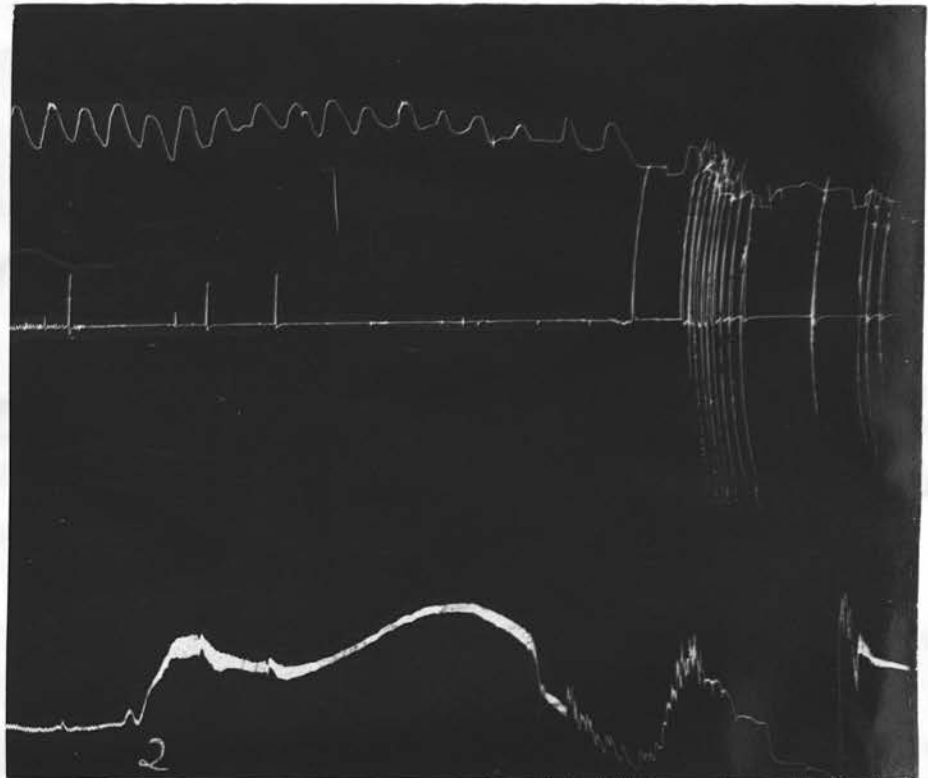


Fig. 1.

Refers to Experiment IV, Table I.

Upper Tracing, - Uterus.

Middle " " - Respiration

Lower " " - Blood Pressure.

At 1, 50 mcms. Histamine introduced into Ileum.

At 2, 10 " " " " Vein.

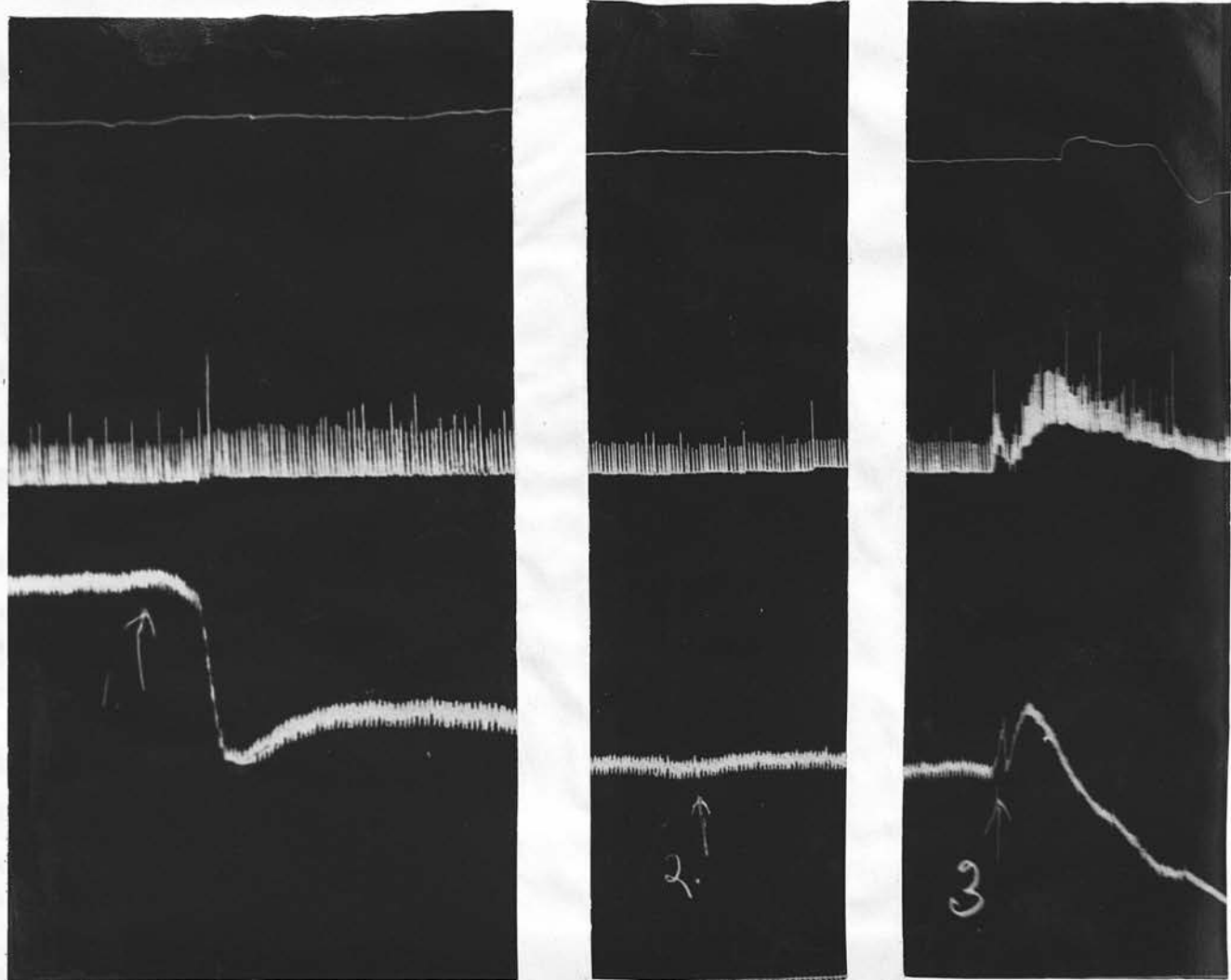


Fig 2.

Refers to Experiment VII, Table I.

Tracings as in Fig. 1.

At 1, 40 mgms. Histamine introduced into Ileum

At 2, 10 " " " "

At 3, 10 " " " vein.

Time-interval between 1 and 2, 15 mins.

" " " 2 and 3, 6 mins.

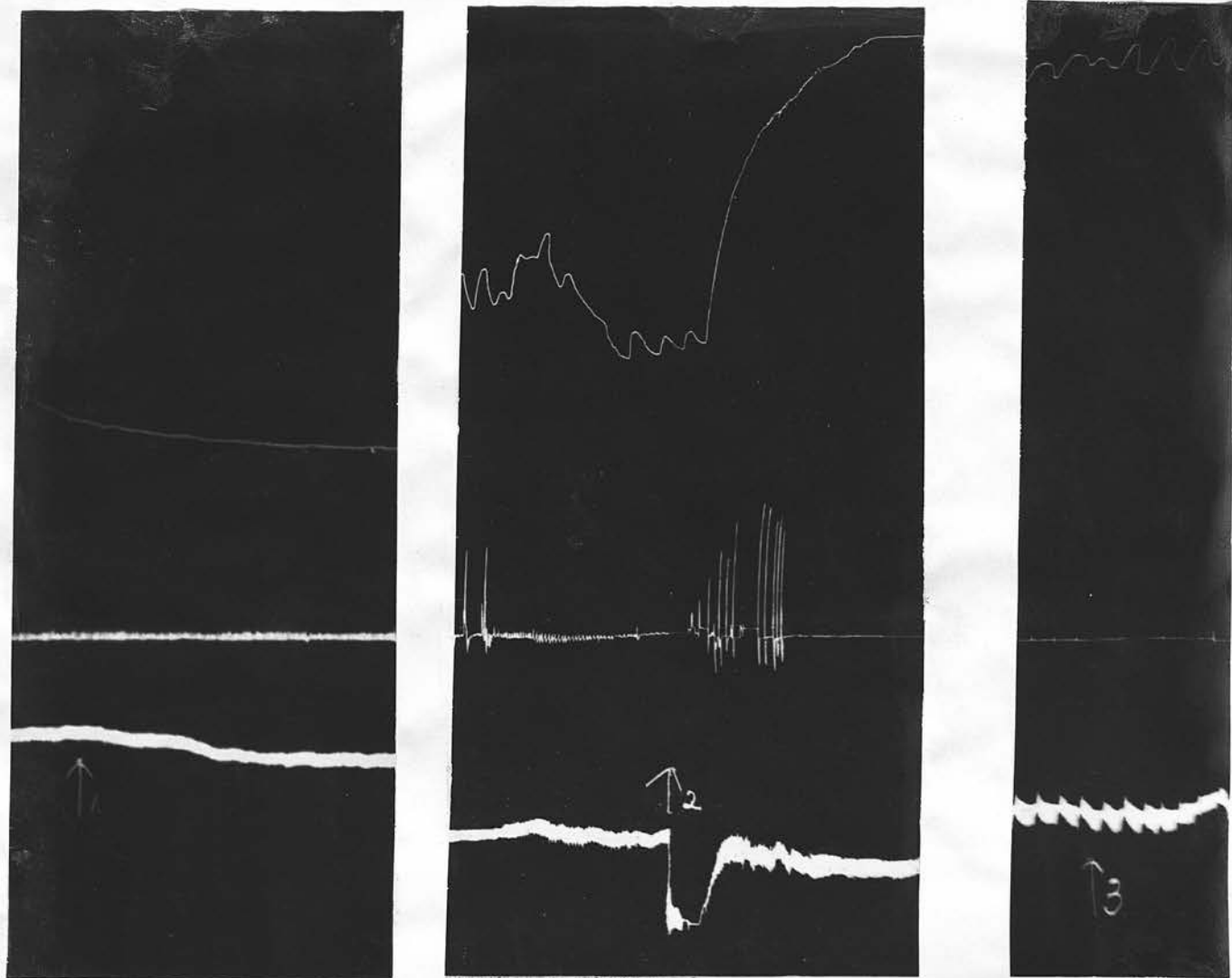


Fig 3.

Refers to Experiment IX, Table I.
Tracings as in Fig. 2.

At 1, 50 mgms. Histamine into Ileum.

" 2, 10 " " " Vein.

" 3, 10 " " " "

Time-interval between 1 and 2, 25 mins.

" " " 2 and 3, 8 mins.

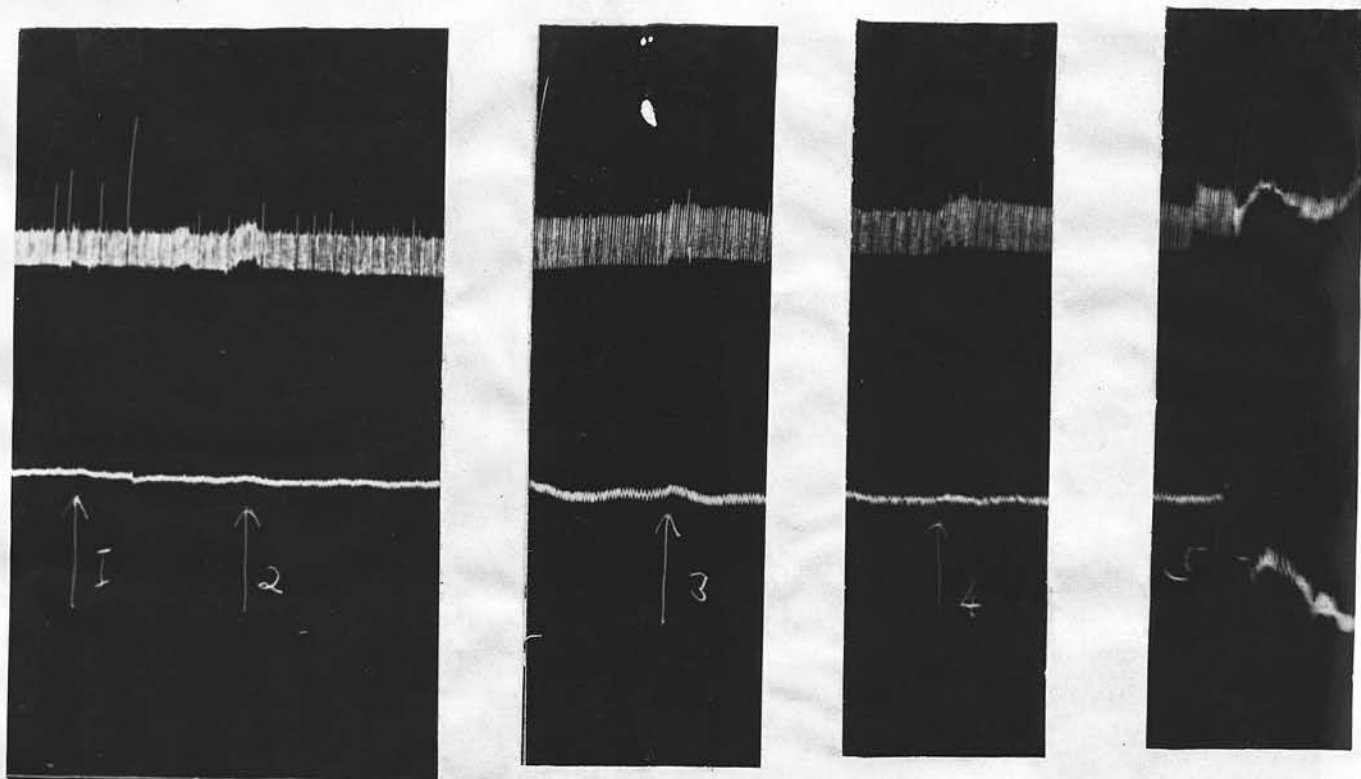


Fig. 4.

Refers to Experiment XIII. Table I.

Upper Tracing - Respiration

Lower " - Blood Pressure.

At 1, 3 c.c. Saline into Caecum.

At 2, 50 mqs. Histamine " "

At 3, 100 mqs. " " "

At 4, 100 " " " "

At 5, 10 " " " Vein

Time-intervals.

Between 2 and 3, 25 mins

" 3 and 4, 12 "

" 4 and 5, 8 "

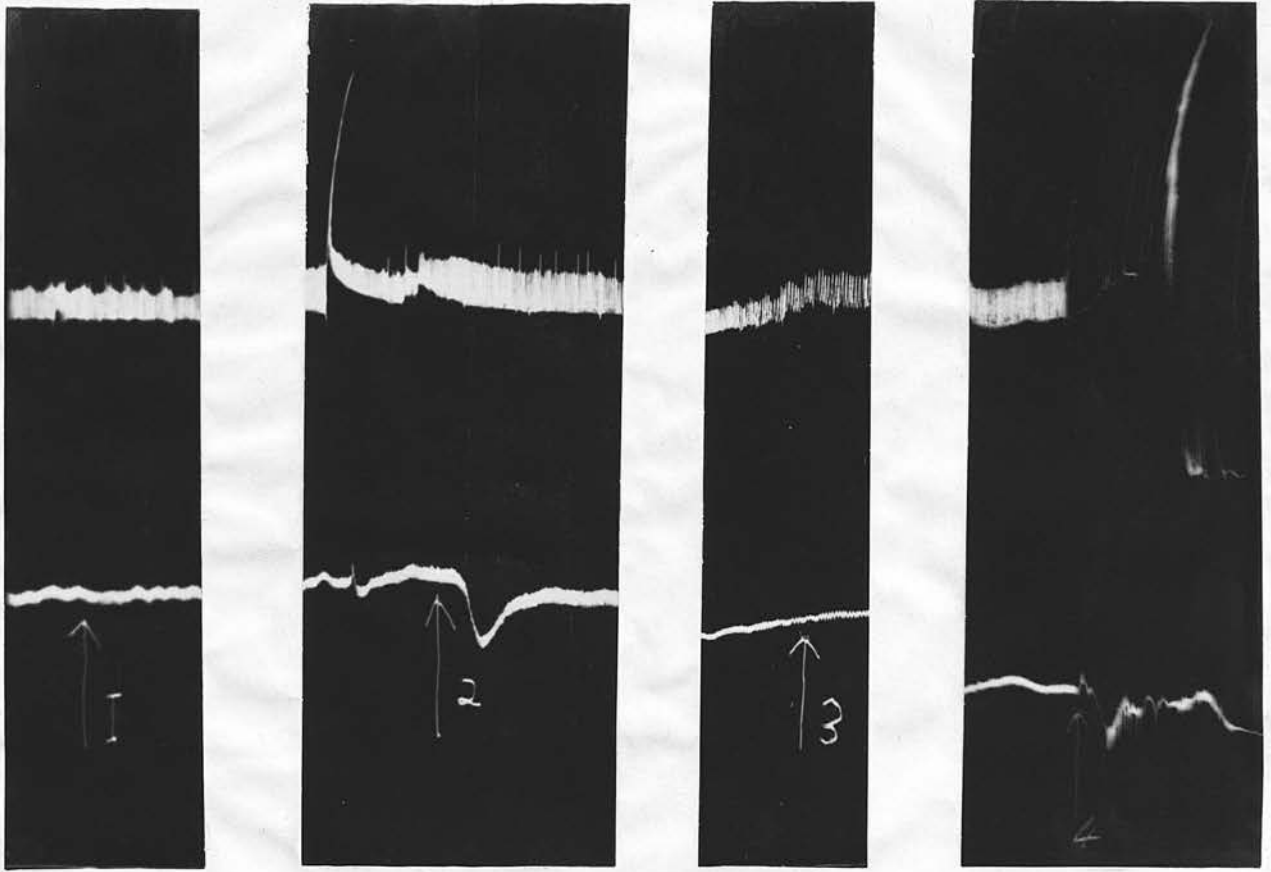


Fig. 5.

Refers to Experiment XIV, Table I.

Tracings as in Fig. 4.

At 1, 5 cc. saline into caecum
 2, 200 mgm. histamine " "
 3, 150 " " " "
 4, 10 " " " vein.

Time-intervals.

Between 1 and 2, 7 mins.
 " 2 and 3, 23 "
 " 3 and 4, 14 "

the third phase may not develop. In those experiments on the ileum where the sharp primary fall of blood pressure does not appear there is a steady decline of the blood pressure of practically the same order as that following the primary fall in the other cases. In the case of the caecum, the steady fall of blood pressure is practically insignificant. It is probable that the sudden primary fall should be a constant feature of all these experiments and that its absence in certain cases may be accounted for by the presence of masses of semi-digested food which delay the process of absorption. Among these cases in which the gut was washed out before the injection of histamine, one only was met with in which the sudden primary fall was absent.

It will be seen from these experiments on the ileum that results obtained under corresponding conditions are not constant; they fall, in fact, into two classes :-

(1) Those exhibiting the sudden primary fall of blood pressure followed by a more or less pronounced recovery which merges into the gradual and persistent decline. This type closely resembles that described by Dale and Laidlaw (6) after intravenous injections of large doses.

(2) /

(2) Those which show the sudden primary fall without rapid recovery, which are analogous to the intravenous injection of small doses as found by Dale and Laidlaw (7).

The fact that there was no evidence of eventual recovery after injection of histamine into the intestine suggests an analogy between the effect produced on absorption and that observed by Dale and Laidlaw (6) on slow intravenous infusion.

The results obtained suggest that the initial effect depends upon the amount of histamine suddenly absorbed which may be a very variable quantity; the subsequent continuous absorption which brings about the slow but persistent fall of blood pressure, is fairly constant for the same part of the intestine; the rate of this continuous absorption must be greater than that of the subsequent destruction or elimination.

It is to be noted that, while the blood pressure is gradually falling, a further injection of histamine into the intestine does not reproduce the acute phenomena, and leaves the steady fall of pressure unaffected, which seems to indicate that

a maximum rate of absorption is reached which is independent of the concentration of histamine in the gut. Intravenous injection of histamine, subsequently to a shock produced by absorption from the gut, produces, instead of the usual fall of blood pressure, a considerable, though transient, rise of the latter. This result is analogous to those obtained by Dale and Richards (8) on adding histamine to the perfusion fluid of isolated organs in the absence of adrenalin or of red blood cells, and is probably due to the effect of the drug in constricting the arteries, unmasked by its effect as a capillary poison.

It has been shown recently by Schenk (9) that the toxic effects of histamine are antagonised by simultaneous doses of adrenalin. The possibility here suggests itself that during slow absorption of histamine, the store of adrenalin in the body, rapidly mobilised to counteract the effect, becomes depleted, and hence at the moment when we give the intravenous injection the conditions prevailing in the body are similar to those obtaining in the above-mentioned experiments of Dale and Richards on isolated organs.

A number of cases of respiratory disturbance were /

were observed during absorption. The earliest response was the development of the periodic type of breathing. If the respiratory effect became more pronounced, it was manifested by a gradual diminution in the depth, with, occasionally, complete cessation of respiration.

The uterus frequently showed an increase of tone during absorption of histamine, particularly when the animal was pregnant.

PART III.

The Effect of Damage to the Intestinal Mucosa upon the Absorption of Histamine.

The experiments described in Part II. show clearly that the absorption of histamine from the normal intestine is a physiological possibility. It remained to find out in what way the rate of absorption /

absorption of histamine was affected by damage to the intestinal mucosa. In order to obtain evidence upon this point further absorption experiments of a similar nature were carried out after such damage had been artificially induced. The Method selected for the production of this damage was the interruption, for 5 to 15 minutes, of the arterial blood supply to the part under investigation; it was considered that this treatment was less likely to introduce disturbing factors than the use of some substance such as sodium fluoride for the purpose.

The results of the experiments by this method are embodied in Table II. and figs. 10 to 13.

TABLE II.

Region of Intestine	No.	Sex	Pregnancy	Weight in grams	Initial dose in mgm.	Dose in mgm. Wt. in grm.	Original B.P. (mm. Hg.)	Shock fall B.P.	Recovery in B.P.	B.P. after 15 mins.	B.P. fall per min.	Remarks
(a) CAECUM	XXV	M.		3850	154	1 : 25	162	42	10	124	2.3	Sup. mesenteric artery clamped 5 minutes
(b) ILEUM					154	1 : 25	124	-	-	104 (10 min)	2.0	
(a) CAECUM	XXVI	M.		1700	68	1 : 25	140	23	13	118	1.5	Artery clamped for 11 minutes.
(b) ILEUM					68	1 : 25	118	43	-	64	3.6	
ILEUM	XXVII	M.		3100	125	1 : 25	158	45	21	115	3.0	Artery off 15 minute
CAECUM	XXVIII	M.		1700	68	1 : 25	108	52	-	37	4.8	Artery off 15 minutes. Artificial respiration.
CAECUM	XXIX	F.	-	1700	68	1 : 25	122	46	19	56	4.4	Artery off 15 minute Artificial respiration.
						With Eck Fistula						
CAECUM	XXX	F.	-	2450	100	1 : 24.5	108	45	-	64	3.0	Artery off 20 min. No absorption of fluid.
ILEUM	XXXI	M.		3250	130	1 : 25	148	70	10	72	5.0	Artery off 15 min. No absorption. Dilution of 1 c.c.

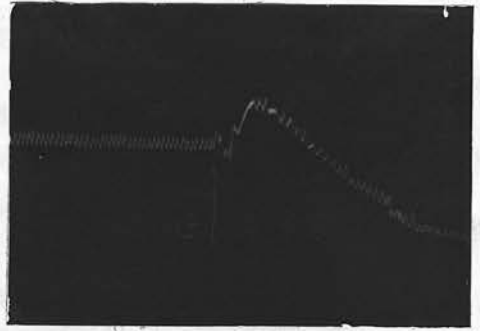
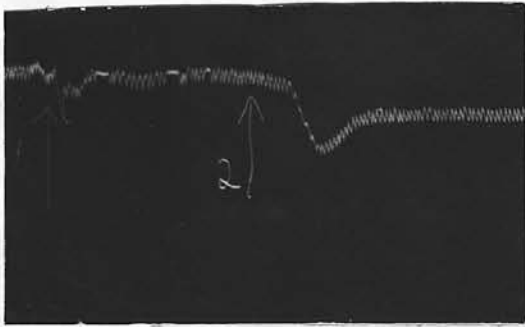


Fig. 10.

Refers to Experiment XXVII. Table II.
 (Artery clamped without Eck fistula)

Tracing of Blood-pressure.

- At 1, clamp removed from artery.
 " 2, 125 mgm. histamine into Ileum.
 " 3, 10 " " " Vein.

Time-interval, Between 2 and 3, 20 mins.

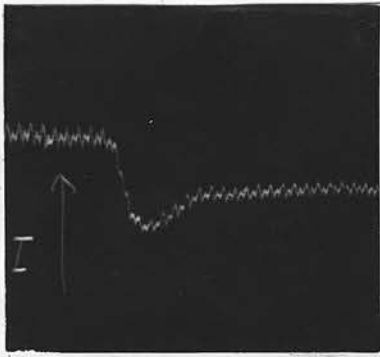


Fig. 11.

Refers to Experiment XXXIX. Table IV.
(Conditions and Tracings as in Fig. 10)

At 1, 68 mgm. histamine into caecum.
" 2, 10 " " " vein.

Time-interval between 1 and 2, 26 mins.

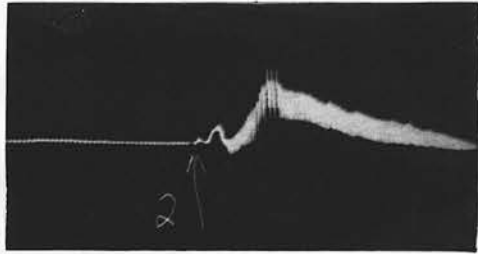
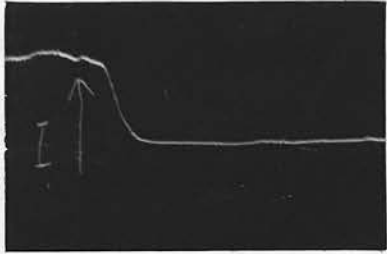


Fig. 12.

Refers to Experiment XXX. Table II.

(Artery clamped and ECR fistula; tracing of blood pressure)

At 1, 100 mgm. histamine into caecum.

" 2, 10 " " " vein.

Time-interval between 1 and 2, 30 mins.

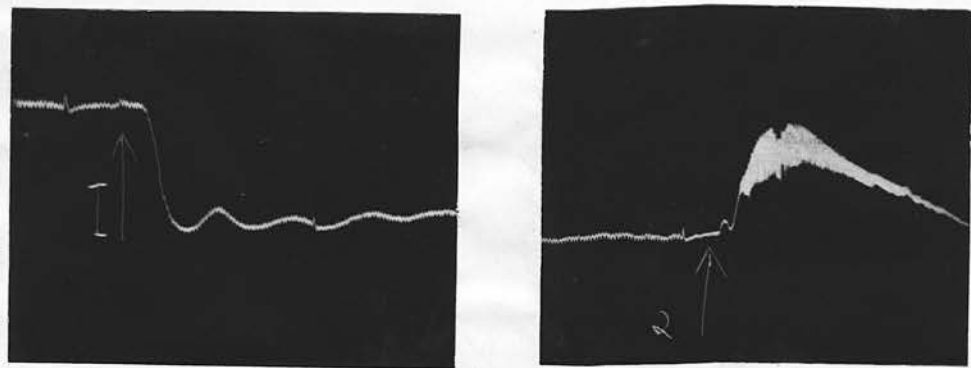


Fig. 13.

Refers to Experiment XXXI. Table II.

(Conditions and tracings as in Fig. 12.)

At 1, 130 mgm. histamine into ileum

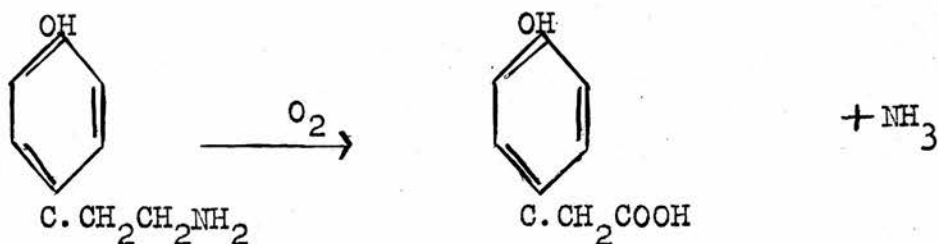
" 2, 15 " " " vein.

Time-interval between 1 and 2, 30 mins.

The outstanding features are the very marked primary fall of blood pressure observed in every case but one, the lack of recovery after this primary fall, and the fact that, after the primary fall, the further fall in blood pressure is extremely slight. This might indicate that under these conditions there was a preliminary absorption of a small amount of histamine, followed by a condition in which absorption practically ceased. There is practically no absorption of fluid; in fact in one or two cases there seemed to be a dilution. This is what one would expect if the mucosa had been reduced to the condition of an ordinary semi-permeable membrane, since the solution injected was distinctly hypertonic. The two caecum experiments which show an abnormal rate of fall of blood pressure are complicated by the fact that artificial respiration had to be maintained throughout; they are therefore scarcely comparable with the other experiments.

PART IV.The Destruction of Histamine by the Liver.

When proof had been obtained of the presence of histamine in the intestine and of the possibility of its absorption therefrom, it became of importance to try and determine whether the body possessed a mechanism by which it was able to destroy this substance or otherwise to protect itself from toxic effects which might be expected to result from its absorption into the circulation. In the cases of tyramine and indole-ethylamine, Ewins and Laidlaw (10) have shown that these substances, on perfusion through the surviving liver are converted to parahydroxy phenylacetic acid and indole acetic acid respectively :-



and that these acids are also found in the urine after oral administration of the respective amines. By analogy therefore, one would expect histamine to be /

be converted by the liver to 4 (or 5) iminazolyl acetic acid which would be an innocuous substance. Dale and Laidlaw (11), however, state that in a few liver perfusion experiments they were unable to observe any destruction of histamine in this organ.

As the available evidence upon this important point did not appear to be very conclusive, further liver perfusion experiments were undertaken. A satisfactory technique was first established and verified by control experiments with substances like glycine which were readily oxidised by the liver under the conditions of experiment. Numerous perfusion experiments with histamine were then undertaken, but the results were entirely negative, confirming the experience of Dale and Laidlaw. As in their case it was found that the physiological activity of the perfusion fluid, as measured by the amount which was required to produce a contraction of the isolated uterus of the virgin guinea-pig, was somewhat increased after perfusion. It was found however, that the substance or substances which gave rise to this increased activity could be destroyed by alkaline hydrolysis. After heating /

heating the perfusion fluid for ten minutes in the boiling water bath with a concentration of 4% of sodium hydroxide and subsequent cooling and neutralisation, the activity of the solution which had been perfused through the liver five times was found to be practically the same as that of the original fluid. It would therefore appear, in view of the results obtained by Ewins and Laidlaw with tyramine and indole ethylamine, that histamine is not readily oxidised in the liver; it is impossible, however, on the basis of perfusion experiments alone, to make a more definite statement since it is obvious that the conditions under which the liver is working, during such experiments, are excessively abnormal, however carefully the conditions are adjusted. A positive result would be conclusive evidence in favour of the possibility of such a change taking place in the organism; a negative result cannot be accepted as final.

In order to obtain a truer and more sensitive indication of the part, if any, played by the liver in the destruction of histamine, it was decided to carry out some further experiments of the type described in Parts II and III, with an
Eck /

Eck fistula in operation. Under these conditions, any histamine which might be absorbed would pass directly into the general circulation, and the effect should therefore be far more prompt than normally, if the liver does play a part in the destruction of the amine.

The procedure of the experiments was as follows :- The first part of the operation was carried out as described in Part II. The inferior vena cava was clamped below the junction of the renal veins, ligatured at its origin from the iliacs and the intervening length stripped free, all small tributories being tied. The vessel was then cut above the ligature and washed out with citrated saline. As long a length as possible of the superior mesenteric vein was then stripped; if it were desired to divert the blood from the caecum or ileum, the vessel was ligatured immediately below its junction with the splenic vein. The superior mesenteric vein was then clamped as far down as possible, cut below the ligature and connected directly with the cut end of the inferior vena cava by means of the method described by Dale and Laidlaw (12). * If the blood from the stomach and duodenum were desired to be diverted, the superior /

superior mesenteric vein was ligatured as low down as possible. It was clamped below its junction with the splenic vein. It was then cut immediately above the ligature and anastomosed with the inferior vena cava; the clamp was then removed and the portal vein ligatured at its entrance into the liver. In this case therefore the blood flow through a short length of the superior mesenteric vein was reversed. The Eck fistula thus arranged was quite satisfactory in the case of the ileum and caecum, but was not so good in the case of the duodenum on account of the fall of blood pressure resulting from the cutting off from the circulation of so large an amount of the gut. This fall in blood pressure was not prevented by the preliminary removal of the lower gut.

TABLE III.

Region of Intestine	No.	Sex	Pregnancy	Weight in grams	Initial dose in mgm.	Dose in mgm. / Wt. in grm.	Original B.P. (mm. Hg.)	Shock fall B.P.	Recovery in B.P.	B.P. after 15 mins.	B.P. fall per min.	Remarks
DUODENUM	XVI.	M.		3300	30	1 : 110	75	22	7	33 (10 min)	4.2	
	XVII.	M.		2750	25	1 : 110	68	10	3	32	2.4	
	XVIII.	M.		3450	100	1 : 34.5	138	46	4	68	5	
	XIX.	F.	+	2100	50	1 : 42	141	-	-	92	2.6	Uterine response
	XX.	F.	+	2400	100	1 : 24	154	68	-	30 (cat dying)	8.2	Respiratory arrest and uterine contractions.
ILEUM	XXI.	F.	-	2500	100	1 : 25	145	69	34	105	2.5	
	XXII.	F.	(recent)	2650	110	1 : 25	108	46	10	66	3	Respiratory and uterine response
	XXIII.	M.		3000	110	1 : 28	102	50	22	48	3.5	Vomiting and respiratory disturbance. Cat dies.
	XXIV.	F.	+	2050	5	1 : 412	128	14	7	121	0.5	Uterine response.

The noteworthy features of the results of the experiments carried out by this method, which are shown in Table III and figs. 6 to 9 are firstly that the Eck Fistula makes little or no difference in the case of the ileum but a conspicuous difference where the caecum is concerned, and secondly that the primary sharp fall in blood pressure is present in practically every case. The reason for the difference in the influence of the Eck Fistula upon the toxic effects of absorption from the ileum on the one hand and the caecum on the other is not obvious. Two possible explanations suggest themselves.

In the first place, Mellanby, in the paper cited above, puts forward the suggestion (for which he was unable to obtain experimental support) that the absorption of histamine may take place largely by means of the lymphatic system. If this were the case the results obtained would be explicable, since the ileum is much more liberally supplied with lymphatics than the caecum; the histamine from the ileum would therefore be absorbed for the most part by the lymphatics and would reach the general blood stream without having to pass through the liver; from the caecum on the other hand it would /

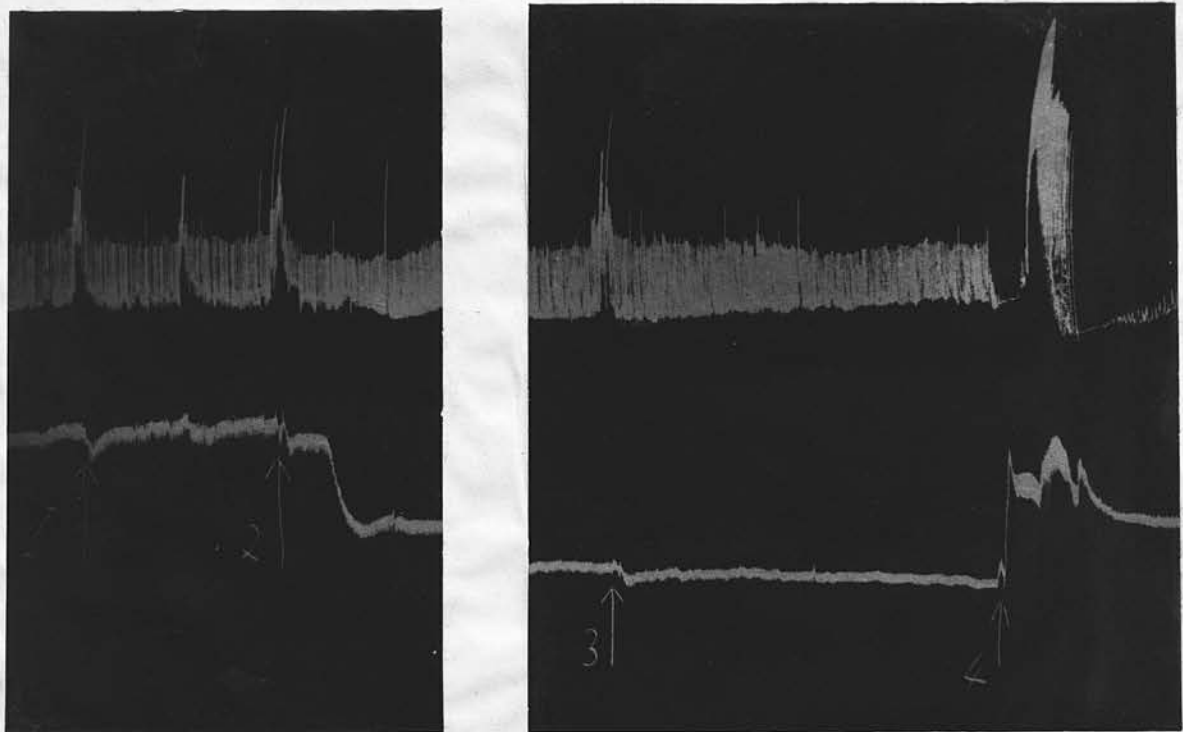


Fig 6.

Refers to Experiment XVIII. Table III.

Upper Tracing - Respiration

Lower " - Blood Pressure.

At 1, 5 cc. saline into Ileum.

" 2, 100 mgm. histamine " "

" 3, 50 " " "

" 4, 15 " " vein.

Time interval between 2 and 3 = 15 mins.

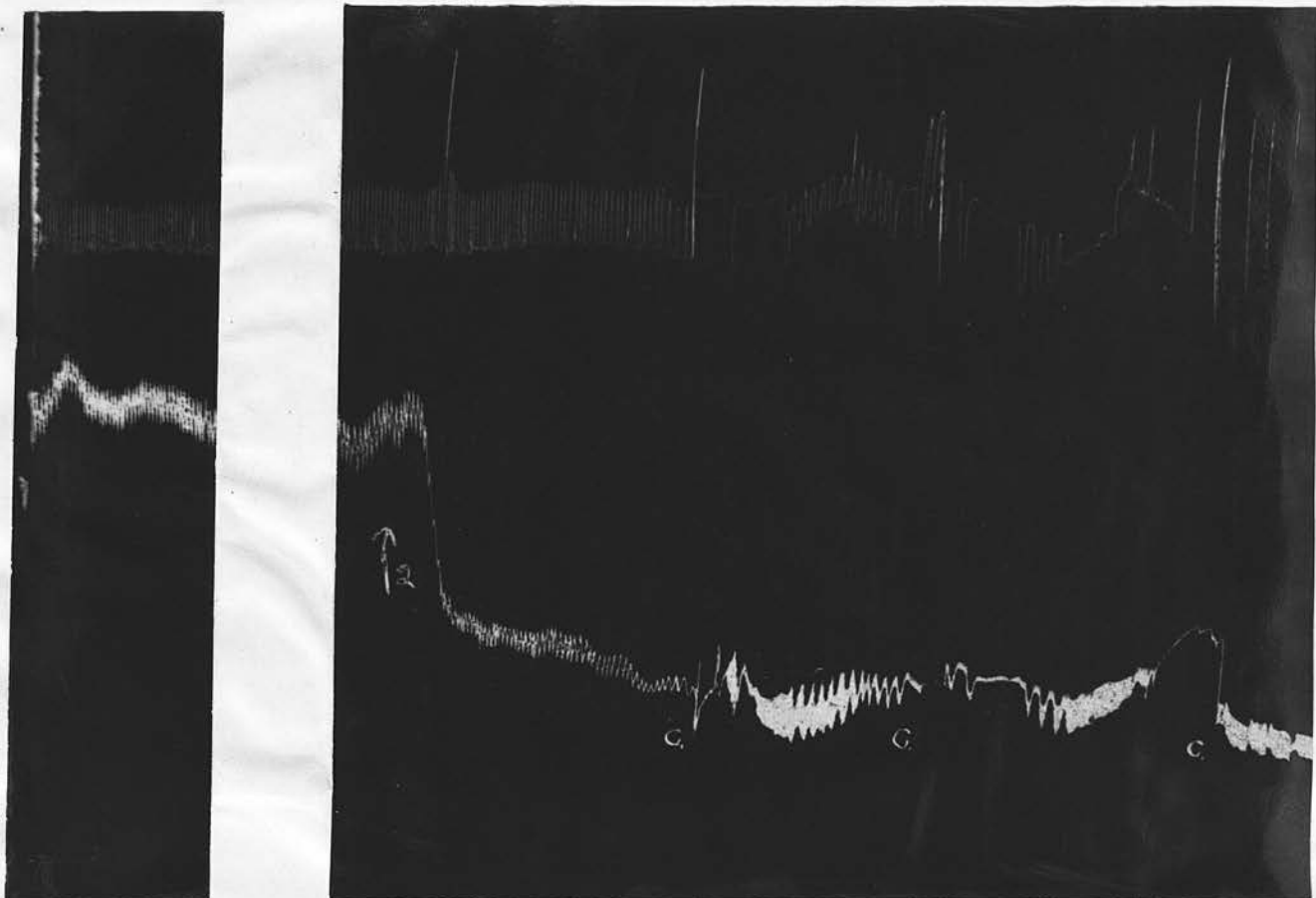


Fig. 7.

Refers to Experiment XX, Table III.

Tracings as in Fig. 6.

At 1, 4 c.c. saline into caecum.

" 2, 100 mgm. histamine " "

Time-intervals.

Between 1 and 2, 7 mins.

" 2 and end, 8 "

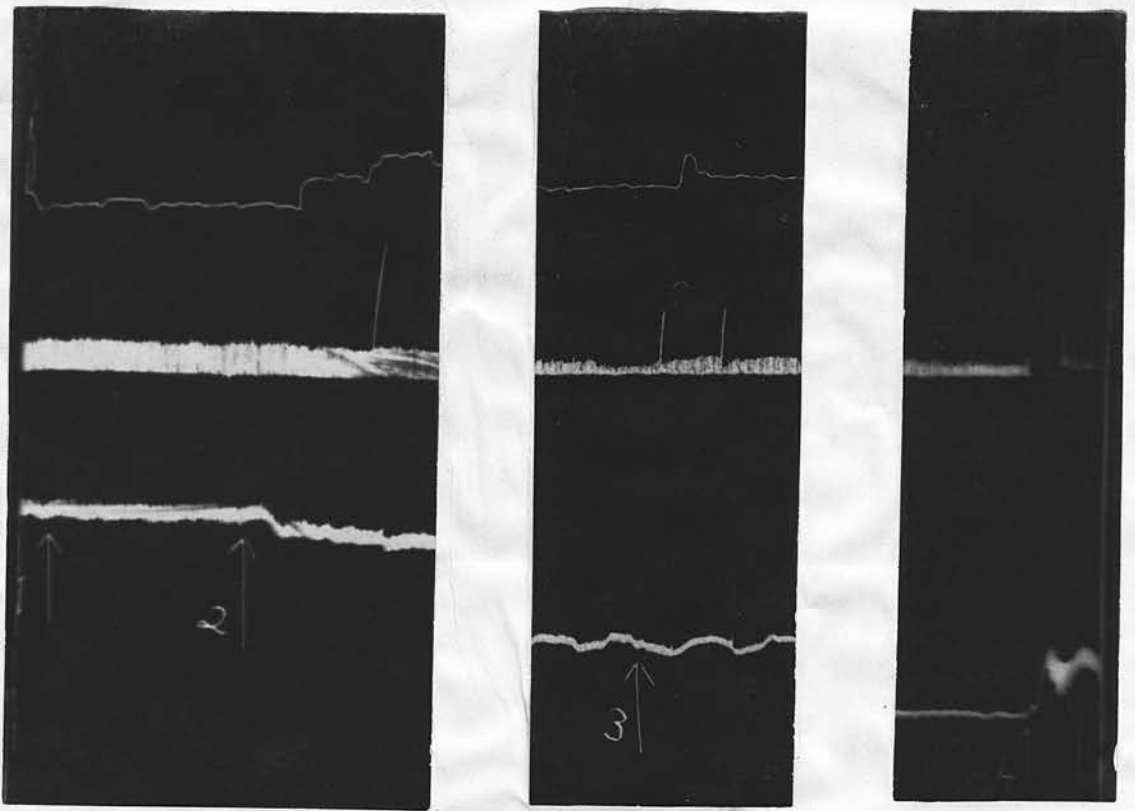


Fig. 8.

Refers to Experiment XIX. Table III.

Upper tracing = Uterus
 Middle .. = Respiration
 Lower .. = Blood-pressure

At 1, 5 c.c. saline into I leum.
 " 2, 50 mgm. histamine
 " 3, 50
 " 4, 10 vein

Time-intervals: - Between 2 and 3, 28 mins.
 .. 3 and 4, 28 ..

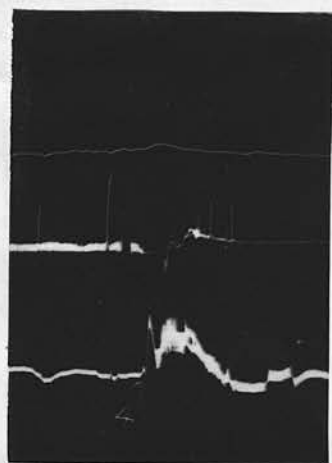
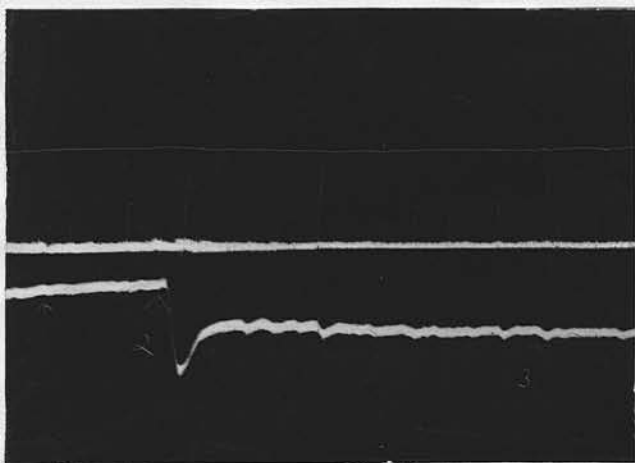


Fig. 9.

Refers to Experiment XXI. Table III.

Tracings as in Fig. 8

- At 1, 5 c.c. saline into caecum.
- " 2, 100 mgm. histamine " " "
- " 3, 50 " " " " "
- " 4, 10 " " " vein.

Time-interval, Between 3 and 4, 65 mins.

would normally be absorbed into the portal system; thus whatever power the liver may have of dealing with histamine would only come into play normally in the case of absorption from the caecum, and it would be in this case alone that an Eck Fistula would make any appreciable difference.

On the other hand, if we assume that there is a maximum rate at which the liver is able to deal with histamine, the phenomenon can be explained by a consideration of the relative rates of absorption from the ileum and the caecum. In Part II it was shown that the effect on the blood pressure of absorption from the ileum was considerably greater than that of absorption from the caecum - that the latter, indeed, was in some cases vanishingly small. Let us assume that the absorption from the caecum is at the rate of x mgms. per unit of time, that from the ileum $x + y$ mgms. per unit of time, and the maximum rate of destruction in the liver z mgms. per unit of time.

From the experiments described in Part II it appears that usually z is very little less than, or equal to x , and both x and z are small in comparison /

comparison with y . Now, under normal conditions, in one unit of time the amount reaching the general circulation from the ileum will be $x + y - z$ mgms; while from the caecum it will be $x - z$ mgms. $x - z$ is a very small quantity and is negligible in comparison with y .

With an Eck Fistula in operation the amounts reaching the general circulation will be $x + y$ mgm. from the ileum and x mgm. from the caecum.

Since $x + y - z$ represents already a fairly large dose the small amount of z added on to it will not produce much more effect; $x - z$ having been however a negligible amount the effect of x in comparison with it will be marked, although x in itself is small.

It seems to the writer that the explanation on the basis of lymphatic absorption cannot be accepted, since absorption by this means would be far too slow to account for the prompt fall of blood pressure which is observed in almost every experiment with the ileum. It seems that some explanation on the lines of the second one suggested is more likely; but for lack of definite evidence the question must be left open for the present.

Either explanation involves the assumption that the liver is dealing with some of the histamine, and indeed, if one considers the results obtained in the case of the caecum, with and without an Eck Fistula, one cannot escape this conclusion. But it is not possible, on the evidence at present available, to ascribe to the liver the power of destroying histamine chemically; it seems probable that the protective function of the liver, as evidenced by the Eck Fistula experiments, may be partially accounted for by the "cushioning" effect of its mass of capillaries which prevents a sudden flood of the toxic substance from reaching the general blood stream.

Summary /

Summary of Results.

- I. Histamine is shown to be present in small amounts in the human intestine (caecum and proximal colon) but cannot be found in the faeces. In some cases the intestinal contents in which histamine was found were obtained after a period of weeks from the date of the operative relief of obstruction.
- II. The rate of absorption of histamine from the normal intestine, as measured by the rate of fall of blood pressure produced, is greatest from the ileum, somewhat less from the duodenum, and very small, though still definite, from the stomach and caecum.
- III. After damage to the intestinal mucosa, produced by cutting off the arterial blood supply for 5 to 15 minutes, absorption occurs at first with a rush, and then almost ceases.
- IV. Although perfusion experiments with the excised liver fail to indicate any destruction of histamine in this organ, absorption experiments with an Eck Fistula in operation seem to show that the liver does exercise a protective function, which may be more mechanical than chemical, against heavy doses of histamine.
-

Discussion.

We may now consider the question of the supposed relation of histamine to one of the types of intestinal intoxication in the light of the results of the experimental investigation described above.

In the first place histamine has definitely been shown to be present in the human caecum and colon but the quantity in which it was found was excessively minute, and, moreover, from the circumstances of some of the cases, it seems very unlikely that its presence was due to any abnormal retention of the intestinal contents. The presumption is therefore strong that histamine is to be regarded as normally present in the intestine.

Since the caecum and proximal colon are the regions of the intestine where bacterial action flourishes, it is in these regions that we may expect to find histamine in the largest amounts; but even massive doses of histamine when introduced into this part of the intestine under experimental conditions produce but little effect, so long as the liver is in the circulation. It is certainly scarcely conceivable that absorption from this region /

region could produce any ill effects, unless, indeed, the conditions of absorption in the human subject are enormously different to those which prevail in cats.

Damage to the intestinal wall certainly does not favour the continuous absorption of histamine; rather does it appear to inhibit it. Were a sudden flood of histamine by some means introduced into a damaged intestine, disastrous results might ensue, but this is a remote contingency and has no bearing on the problem at present under consideration.

We are left, then, with one remaining possibility - the absorption from the ileum.

It has unfortunately been impossible to obtain ileal contents for examination but it does not seem likely that, normally, histamine should be formed to any great extent in this part of the intestine. If, however, there should exist incompetency of the ileo-caecal valve, there is the possibility that caecal contents containing histamine might be re-gurgitated into the ileum and that from this region the histamine might be absorbed almost quantitatively.

This seems to the writer to be the only conceivable /

conceivable condition of affairs in which histamine could act as a toxic agent; and even in this case, unless the amount present were very much larger than would seem to be indicated by the results given in Part I, above, or unless, for some reason, the liver were out of action as a protective mechanism, it is difficult to imagine that ill effects could be produced. The balance of evidence at present therefore seems to be distinctly against the assumption that histamine is an active agent in causing the symptoms of intestinal intoxication; if it ever is so, it can only be in those cases where a definite deficiency, either structural such as ileo-caecal incompetence, or functional such as liver insufficiency, is involved; the mere presence of histamine in the intestine, without other contributing factors, cannot account for the symptoms observed.

Note -

The work described above forms the substance of two papers, written by the author in conjunction with Prof. J.C. Meakins, which are at present in course of publication in the Journal of Pharmacology and Experimental Therapeutics.

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