J. Physiol. (1960), **153**, *pp.* 239–249
With 1 *text-figure*
Printed in Great Britain

TISSUE 5-HYDROXYTRYPTAMINE AND URINARY 5-HYDROXYINDOLEACETIC ACID AFTER PARTIAL OR TOTAL REMOVAL OF THE GASTRO-INTESTINAL TRACT IN THE RAT

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(*Received* 11 *January* 1960)

It is generally accepted that the gastro-intestinal mucosa represents the most important site of production and storage of 5-hydroxytryptamine (5-HT) in mammals. In the rat, the total 5-HT in the gastro-intestinal tract may vary according to age, weight, strain, diet, and probably season, between 80 (Erspamer, 1954) and 300 μg/kg (Bertaccini, unpublished). Of this total amount, about 8–15 % is contained in the stomach, 30–40 % in the small intestine and the remaining 45–60 % in the large intestine.

On the basis of these data and the easy availability of a large number of uniform animals, rats seemed particularly suitable for studying the effect of removal of important portions of the 5-HT-secreting intestinal tissue or of the entire gastro-intestinal tract, on the serum and tissue levels of 5-HT as well as on the urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of 5-HT.

METHODS

Experimental animals. The large intestines were removed from three groups of rats (300 in all) weighing 180–250 g. Each group consisted of animals of the same breed and sex, and of approximately the same age. The first experiment was carried out on male rats kept on a non-standard laboratory diet; the second on female rats, and the last on male Wistar rats. In the second and third experiments the rats were kept on the Rockland diet. Complete gastro-enterectomy was performed on 60 rats of both sexes; 30 rats were kept as controls.

Operation procedure and post-operative treatment

Removal of the large intestine. The rats were starved 24 hr before operation, but were allowed water *ad lib.*

The well known resistance of rat's peritoneal cavity against infections allowed the operation to be performed with care for cleanliness but without rigorous asepsis. The operative area was shaved and painted with 5 % tincture of iodine U.S.P.; penicillin 10,000 u./rat was given subcutaneously for 1 day before and 2 days after the operation, to reduce the risk of peritonitis due to leakage at the site of anastomosis.

Under light ether anaesthesia the abdomen was opened by a mid-line incision and the appendix was delivered from the peritoneal cavity on to saline packs. Beginning from the ileocaecal valve, all the blood vessels supplying the portion of the intestinal tract that was to

be excised were ligated. The large intestine was separated from its mesentery by careful dissection up to a point as near as possible to the anus, and the gut without its mesentery was thus gently excised with the minimum trauma and stretching. After the resection the continuity of the bowel was restored by an end-to-end anastomosis, with fine silk and a small curved needle. Side-by-side anastomosis was found to be more difficult to perform and less well tolerated. The abdomen was then closed with four or five interrupted sutures. Catgut was used for muscle layers and silk thread for skin. Some of the controls were subjected to simple laparotomy.

After the operation the animals were housed, in groups of two or three, in dry, clean cages. Twice in the first 24 hr 6 ml. of saline solution with 5 % glucose was administered subcutaneously to each rat. From the second day on animals were offered milk and sugar, soaked bread and fresh liver in addition to amino-acid and vitamin mixtures. The rats were returned to their usual diet a fortnight after the operation. Intact and laparotomized control rats were always given the same diet as the operated ones.

The post-operative course was very satisfactory and the survival rate was 70–75 %. The only trouble was a diarrhoea which lasted for about 1 month. Thereafter the stools became more formed. All animals lost weight during the first month, but then resumed normal growth rates comparable to, and in some instances greater than, in the controls. Operated rats showed, after recovery, a striking voracity.

As in the experiment of Clatworthy, Saleeby & Lovingood (1952), who examined dogs deprived operatively of 40–80 % of the small intestine, Bertaccini, Nobili & Zamboni (1959) found that 3 months after the operation there was a significant rise in haemoglobin concentration and erythrocyte and platelet counts. In addition, operated rats presented a 35 % increase in oxygen consumption above that of control rats and a slight rise (+ 0·6° C) in body temperature.

The animals were killed by decapitation at varying intervals after the resection. The viscera appeared quite normal and, except in rats operated 10 and 15 days before, no traces of intestinal suture were found. However, the stomach plus small intestine of operated rats showed a weight increase of 10–20 % compared with the controls.

Total gastro-enterectomy. The animals were not starved before operation. Under ether anaesthesia a long, mid-line incision was made and wide exposure was obtained by the use of a retractor. First, gastrectomy was performed as described by Meli (1957). After this the bile duct was delivered and gently separated from mesentery and duodenal vessels. A small portion of mucosa of the duodenal loop including the outlet of the bile duct was isolated between clamps, severed and then inserted into the abdominal wall, to drain the bile externally. A single ligature was placed round the coeliac and the superior mesenteric arteries as near the aorta as possible. The rectum with its vessels was clamped, ligated distal to the clamp, and then cut between the clamp and the point of ligation. Finally, the whole gut, freed from its ligamentous and vascular attachments, was removed.

In some groups of rats, to avoid the contamination of urine from bile, which could interfere with the estimation of 5-HIAA, the bile duct was left free in the abdomen. After removing the gastro-intestinal tract, the abdomen was filled with sterile gauze. Penicillin 10,000 u./rat was given immediately after the operation, and 6 ml. of physiological saline solution with 5 % glucose was injected subcutaneously every 6 hr. Because of the severity of this operation only 40–50 % of the animals could be used for experiment. They were killed by decapitation 1–3 days later.

Analytic procedure

Preparation of acetone extracts. The following tissues and organs were examined: blood, gastro-intestinal tract, spleen, brain, lungs, liver, kidneys, ears and hind paws.

Blood was kept for 2–3 hr at room temperature, then during the night at + 4° C. The serum was separated by centrifugation and then treated with 4 vol. of acetone. After standing during the night the liquid was filtered and the clear filtrate used for bioassay.

The gastro-intestinal tract was opened longitudinally, washed under running tap water and carefully dried with filter paper. It was minced with scissors and extracted with 4 parts (4 ml./g) of acetone for 24 hr, and then, after decanting the liquid, re-extracted for another 24 hr with 3–4 parts of 80 % acetone. The combined filtrates were stored in the cold until used. All other tissues were treated in the same way but without washing. Before the bioassay acetone was removed by evaporation under reduced pressure at 40–50° C, or under an air stream in a boiling water-bath. The remaining aqueous liquid was brought to the desired volume with physiological saline solution.

Bioassay of 5-HT. Ovariectomized adult rats were injected once or twice with 50–100 μg oestradiol dipropionate 4–7 days before they were used. Uteri from these animals were suspended in a 10 ml. bath of atropinized (10^{-7}) Tyrode solution, kept at 30° C and bubbled through with air. The period of contact between uterus and the active solution (5-HT or tissue extract) was for 3–4 min and the interval between doses was 2–3 min. In the assays particular attention was paid not only to the heights of the contractions but also to how well they were maintained (Erspamer, 1940). The standard was 5-HT creatinine sulphate (Farmitalia S.p.A., Milan); the results, however, were always expressed in terms of 5-HT base.

Urine collection and estimation of urinary 5-HIAA. Groups of four rats were put into diuresis cages. Urine was collected in graduated cylinders containing 0·3 ml. of chloroform and 0·3 ml. of acetic acid, in order better to preserve the excreted 5-HIAA. At the end of the collection period the urine was either immediately frozen or treated with 1 vol. acetone which, of course, had to be removed by evaporation before the estimation of 5-HIAA. The acetone-urine was stored at −15° C. In preliminary experiments it was found that the content of 5-HIAA remained unchanged for at least 1 week in frozen urine, and for up to 15 months in urine plus acetone kept at −15° C. The 5-HIAA in urine was estimated by the colorimetric method of Macfarlane, Dalgliesh, Dutton, Lennox, Nyhus & Smith (1956).

Chromatographic detection of tryptophan and its metabolites. This was carried out in control as well as in operated rats after a loading dose of L-tryptophan (100 mg/kg by gastric tube). After the tryptophan administration the animals were housed in metabolism cages and stools and urine were collected separately for 24 hr. Faeces were weighed and extracted with 4 parts (4 ml./g) of acetone; urine, reasonably free from faecal contamination, with 1 part of acetone.

The concentrated extracts were chromatographed ascendingly on Whatman No. 1 paper. For unidimensional chromatograms the solvent was the *n*-butanol–acetic-acid–water mixture (4:1:5); for bidimensional chromatograms the same mixture was used for the first run and distilled water for the second. Tryptophan and other indoles were visualized by spraying with a 2 % alcoholic solution of *p*-dimethylaminobenzaldehyde followed by exposure in a large glass chamber to HCl vapour; kynurenine and xanthurenic acid were visualized by their fluorescence reaction (Coppini, Benassi & Montorsi, 1958).

RESULTS

Tissue levels of 5-HT after removal of the large intestine. The results on 24 groups of rats are summarized in Table 1. The tissue contents of 5-HT were followed for up to 7 months after removal of the large intestines. In two other experiments carried out in different seasons, with male rats of different strain, results were obtained in substantial accordance with those shown in Table 1. Particular emphasis should be laid on the fact that brain 5-HT levels regularly presented a 20–30 % decrease following removal of the large intestine.

TABLE 1. 5-HT content (μg/g or μg/ml.) of some tissues of the rat after removal of the large intestine. Each experiment was done on the pooled tissues from 4 rats. When an average is given the number of experiments is shown in brackets

	Days after the operation										
Tissue	Control 0*	10	15	30	Control 60†	60	90	Control 120†	120	Control 225†	225
Brain	0·26		0·23	0·25							
	0·27	0·17	0·24	0·32	0·50	0·22	0·25		0·32	0·36	0·20
	0·32	0·16	0·20	0·18	0·41	0·28	0·36	0·33	0·24	0·31	0·21
	0·30		0·21	0·25	0·36					0·29	
	0·30										
Stomach and small intestine	2·51 (6)	2·27 (2)	2·34 (4)	1·87 (4)	2·30 (3)	1·73 (2)	2·28 (2)	3·70	2·70 (2)	3·08 (2)	2·05
Serum	0·59 (6)	0·63 (2)	0·51 (4)	0·62 (4)	0·53 (3)	0·54 (2)	0·43 (2)	0·60	0·66 (2)	0·82 (3)	0·85 (2)
Spleen	2·25 (5)	2·60 (2)	1·67 (4)	2·05 (4)	2·96 (3)	2·70 (2)	2·79 (2)	1·93	1·79 (2)	2·43 (3)	2·00 (2)
Lungs	2·14 (6)	2·00 (2)	1·93 (3)	1·56 (4)	1·93 (3)	2·16 (2)	3·71 (2)	2·88	2·95 (2)	3·35 (3)	3·00 (2)
Liver	0·35 (3)	0·30 (2)	0·24 (2)	0·15 (2)	0·27 (3)	0·32 (2)	0·36 (2)	0·35	0·28 (2)	0·45 (3)	0·37 (2)
Kidneys	0·10 (2)	0·11 (2)	0·12 (2)	0·05	0·09 (2)	0·05	0·19 (2)	0·15	0·18 (2)	0·15 (2)	0·18
Ears	0·32 (2)	0·43	0·31 (2)	0·63 (2)	0·67 (3)	0·79 (2)	0·68 (2)	0·43	1·06 (2)	0·33 (3)	0·94 (2)
Paws	0·15 (5)	0·22 (2)	0·17 (4)	0·15 (4)	0·19 (3)	0·39 (2)	0·40 (2)	0·44	0·44 (2)	0·19 (3)	0·24 (2)

* Control rats killed at the beginning of the experiment. † Control rats killed 60, 120 and 225 days, respectively, from the beginning of the experiment.

The following conclusions may be drawn from the tabulated data:

(1) The 5-HT content of stomach and small intestine did not show significant changes at any time after operation. The observed fluctuations in the 5-HT levels were always within the normal range. In this respect it should be noted that the slight diminution in the 5-HT content, per gram of fresh tissue, of the small intestine of operated rats is compensated for by the 10–20 % increase in the weight of the small intestine observed in these animals compared with the controls.

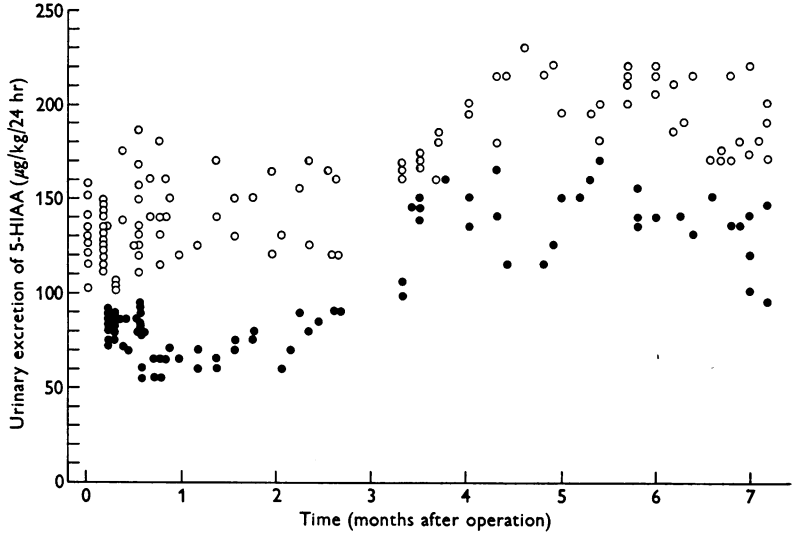

Fig. 1. Daily urinary excretion of 5-HIAA after removal of the large intestine; ○, controls, ● operated rats. Each value refers to the pool of urine obtained from four rats.

(2) The same may be said for serum and all other examined tissues except the brain. In some cases (e.g. lungs, liver, kidneys) a tendency to a lowering of 5-HT levels was observed, especially 15 and 30 days after the operation, but, owing to the considerable variations in the normal 5-HT content of rat tissues, it was impossible to ascribe any definite significance to the above occasional changes.

(3) The 5-HT content of the brain was significantly lower ($P < 0{\cdot}01$) in operated animals than in controls.

5-HIAA excretion after removal of the large intestine. The results are shown in Fig. 1. The daily urinary excretion of 5-HIAA was determined at the beginning of the experiments both in control rats as well as in the rats which were later to be submitted to removal of the large intestine. Each value refers to the pool of urine obtained from four rats. It is clear from Fig. 1, which illustrates an experiment lasting more than 7 months,

that removal of the large intestine regularly produced a conspicuous decrease in the daily urinary excretion of 5-HIAA. This decrease was already evident in the first days after the operation and reached its maximum during the first 2 months, the values of urinary 5-HIAA being in the operated animals only 50 % of those found in the controls. It may also be seen from Fig. 1 that, whereas the values of 5-HIAA in control rats were rather scattered, those in operated rats were much less so. Later on, starting from the third month, the urinary excretion of 5-HIAA began to rise both in control and in operated animals. A maximum increase was reached in the fifth month, then the values tended to remain constant until the end of the experiment. It may be noted, however, that the percentage increase in the operated animals was more marked than that in the controls. In fact, whereas operated rats were excreting 65 μg/kg/24 hr on the 15th–60th days after the operation and 140 μg/kg/24 hr 4 months after the operation (more than 100 % increase) control rats were excreting 145 μg/kg/24 hr and 200 μg/kg/24 hr respectively (38 % increase). It is uncertain whether the general increase in 5-HIAA excretion, observed in the control rats during the course of our experiments, is due to the ageing of the animals (old rats contain in their tissues more 5-HT than young animals; Bertaccini, 1958*b*) or whether it is to be ascribed to seasonal variation.

Absorption of dietary tryptophan (TP) in normal and operated animals. L-tryptophan is the remote precursor of 5-HT, and hence of 5-HIAA. A deficiency in dietary TP produces in human beings and animals both a reduction in tissue 5-HT and in urinary 5-HIAA; an excess of dietary TP has the opposite effect (Stacey & Sullivan, 1957; Eber & Lembeck, 1958; Zbinden, Pletscher & Studer, 1958; Towsend, Katis & Sourkes, 1958).

It is well known that TP absorption takes place exclusively in the small intestine. Nevertheless, it seemed necessary to exclude the possibility that the reduced urinary 5-HIAA observed in our operated rats was at least partly due to a diminished intestinal absorption of TP. A number of rats were therefore given a dose of L-TP 100 mg/kg by stomach tube. It was possible to demonstrate by paper chromatography that absorption and metabolism of the amino acid were the same both in control and in operated animals. In fact, only traces of the administered TP were found in the stools and very small amounts in the urines. There were also no appreciable differences in the urinary excretion in xanthurenic and kynurenic acids between operated and control animals, as assessed by visual comparison, in Wood's light, of bidimensional chromatograms of urine. Since significant differences between control and operated rats could not be found in 5-HT content of the small intestines, the serum and other tissues (except brain), it may be concluded that the reduction in urinary

excretion of 5-HIAA after removal of the large intestine was not in any way due to deficiency in TP absorption or metabolism.

Tissue levels of 5-HT after removal of the entire gastro-intestinal tract. The results obtained are summarized in Table 2: in each experiment tissue from two to four rats was pooled. The main conclusions which may be drawn from the tabulated data are as follows: (*a*) In spite of acute removal of all the 5-HT-secreting cells of the gastro-intestinal mucosa, there was no appreciable change in 5-HT content in the brain, and in the ears and paws. (*b*) Serum, spleen and lungs showed a marked decrease in 5-HT content.

TABLE 2. 5-HT content (μg/g or μg/ml.) of some tissues of the rat after removal of the entire gastro-intestinal tract. Each experiment was done on the pooled tissues from 2–4 rats. When an average is given the number of experiments is shown in brackets

		Operated animals, days after operation		
Tissue	Controls	1	2	3
Brain	0·33 (5)	0·24	0·33 (5)	0·26
Serum	0·82 (3)	0·43	0·27 (2)	0·14
Spleen	2·40 (5)	2·00	1·43 (5)	1·30
Lungs	3·00 (5)	1·60	1·50 (5)	0·76
Ears	0·35 (5)	1·20	0·57 (4)	—
Paws	0·25 (5)	0·22	0·29 (4)	0·13

5-HIAA excretion after removal of the entire gastro-intestinal tract. The results shown in Table 3 demonstrate that removal of the entire gastro-intestinal tract produced a tremendous decrease in the urinary excretion of 5-HIAA. In the first 24 hr period the urine contained barely 10% of the normal 5-HIAA, in the second 24 hr period less than 3–4%. Urine volume was of course greatly reduced, but the urinary excretion of 5-HIAA in a 24 hr period has been shown to be largely independent of the quantity of urine: in fact hydrated rats and rats deprived of water excreted approximately the same amount of 5-HIAA.

Urinary excretion of 5-HIAA following tryptophan administration in gastro-enterectomized rats. As was previously stated, gastro-enterectomy produced a striking reduction in the urinary excretion of 5-HIAA. It then seemed worth while to establish whether this was due only to removal of the gastro-intestinal 5-HT-secreting tissue or also to lack of L-TP in other tissues which may be capable of a complete synthesis of 5-HT, e.g. brain tissue and mast cells. Twelve operated rats were therefore given 4 subcutaneous doses each of L-TP 100 mg/kg, the first immediately after operation, and then 4, 8 and 12 hr later. Eight intact rats were subjected to the same treatment. Intact rats, and operated rats which were not given tryptophan, acted as controls.

It was observed that the administration of L-TP, while causing a slight

increase (up to 20%) in the intact rats, did not produce any increase in the urinary 5-HIAA of operated rats, which excreted < 10% of the normal 5-HIAA. Hence, it does not seem probable that lack of L-TP had played any important part in the reduction of urinary 5-HIAA observed in gastro-enterectomized animals.

TABLE 3. Daily urinary excretion of 5-HIAA (μg/kg) after removal of the entire gastro-intestinal tract. Each value refers to the pooled urine obtained from 2–4 rats

Before operation		After operation	
		0–24 hr	25–48 hr
200	210	14	6
190	170	12	4
185	220	11	4
205	195	26	4
190	—	12	—
158	—	27	—
210	—	18	—
150	—	15	—
182	—	12	—
170	—	16	—
Means 184	198	16·3	—

DISCUSSION

The present experiments have demonstrated that removal of the large intestine in rats, while leaving virtually unaffected the 5-HT content of nearly all the tissues examined, produced a conspicuous decrease in the 5-HIAA of the urine. Because no alterations in the absorption and metabolism of dietary L-TP and consequently no deficiency of L-TP, could be demonstrated, it is highly probable that the reduction in urinary 5-HIAA excretion was due to a diminished 5-HTP (5-hydroxytryptophan) and 5-HT biosynthesis, caused by a reduction of the 5-HT-secreting tissue in the gastro-intestinal tract, namely the enterochromaffin cells. The percentage decrease in urinary 5-HIAA excretion, especially in the first 2 months after the operation, agrees satisfactorily with the reduction in the 5-HT-secreting tissue provoked by removal of the large intestine. The obvious conclusion is that biosynthesis of 5-HT is carried out mainly in the mucosal enterochromaffin cells, and that urinary 5-HIAA is derived mainly from 5-HT of intestinal origin, as is well known (Erspamer, 1940; Toh, 1954; Dalgliesh & Dutton, 1957*a*, *b*; Haverback & Davidson, 1958; Bertaccini, 1958*a*; Rosenberg, Davis, Moran & Zimmermann, 1959; Erspamer & Testini, 1959; Bertaccini & Chieppa, 1960). Total gastro-enterectomy produced a virtual disappearance of urinary 5-HIAA, in spite of repeated parenteral administration of very high doses of L-TP. This represents strong additional evidence in favour of the hypothesis that 5-HT is the specific hormone of the entero-chromaffin cell system

(Erspamer & Asero, 1952), and proves also that, under normal conditions, practically all urinary 5-HIAA is in fact derived from the 5-HT of intestinal origin.

Removal of the large intestine, which, as already stated, might cause even a 50 % reduction in the urinary excretion of 5-HIAA, did not produce any significant change in the 5-HT content either of blood serum (i.e. platelets) or of other tissues, except brain. Thus, in spite of reduced 5-HT secretion by the enterochromaffin cells, platelets while passing through the small intestine absorb from the plasma enough 5-HT to maintain their normal content of 5-HT. It is well known that they can absorb 5-HT against a gradient of 170/1 (Stacey, 1956). In fact, the 5-HT level of the part of the gastro-intestinal tract which was left *in situ* after operation was practically normal. This is in sharp contrast with the decreased 5-HT content of the gastro-intestinal mucosa in rats on a low-tryptophan diet (Eber & Lembeck, 1958).

Since liver, lungs, ears and paws of operated animals showed a practically normal level of 5-HT, it is evident that in these tissues too a normal absorption of 5-HT from blood or, more likely, a normal biosynthesis of 5-HT, has taken place, also in spite of a reduced biosynthesis of the amine by the enterochromaffin tissue. It should be noted that in the above rat tissues 5-HT is mainly localized in mast cells, which synthesize 5-HT from L-TP (Schindler, Day & Fischer, 1959).

Total gastro-enterectomy produced a significant decrease in the 5-HT content of blood serum, spleen and lungs, but not a total disappearance of the substance, as in the case of urinary 5-HIAA.

Removal of the large intestine provoked in the rat a significant lowering of the 5-HT content of brain tissue. The reason for this remains largely obscure. It may be that the decrease of brain 5-HT is somehow correlated with the increase in basal metabolism and body temperature observed by Bertaccini *et al.* (1959) in operated animals. Gastro-enterectomized rats, even when killed 2–3 days after operation in extremely bad condition, showed a virtually normal brain 5-HT content. The most likely interpretation of this fact is that brain tissue carries out a normal biosynthesis of 5-HT independently of the gut. Other possibilities are not easy to accept considering that the half-life of brain 5-HT is believed to be approximately 10–30 min (Udenfriend & Weissbach, 1958), so that in the 48-hr survival period the cerebral 5-HT ought to have been renewed 50–150 times.

Although the results obtained in the present investigation are, on the whole, in favour of the hypothesis that brain 5-HT is completely of local origin, other alternatives should be kept in mind. In fact, besides the intestine, the rat contains another important source of 5-HT in the mast

cells. Under these circumstances it is evidently impossible to decide whether the 5-HT found in the brain after total gastro-enterectomy is produced in the brain itself from L-TP, or whether it is derived by decarboxylation of 5-HTP offered to the cerebral tissue by the mast cells.

SUMMARY

1. Removal of the large intestine and total gastro-enterectomy have been carried out in rats with the purpose of investigating the effects of reduction or absence of the 5-hydroxytryptamine-secreting enterochromaffin cells on the biosynthesis of 5-hydroxytryptamine (5-HT).

2. Removal of the large intestine produced a considerable decrease in the daily 5-hydroxyindoleacetic acid (5-HIAA) urinary excretion, independent of disturbances in tryptophan absorption of metabolism. The 5-HT content of blood and other tissues examined did not show any appreciable change. The only exception was the brain, the 5-HT level of which was reduced.

3. Total gastro-enterectomy caused a virtual disappearance of 5-HIAA from the urine and a considerable lowering of the 5-HT in blood, spleen and lungs. No change in the 5-HT level was observed in the brain, paws and ears.

4. The enterochromaffin cells of the gastro-intestinal mucosa therefore represent the main source of 5-HT in the rat and practically the only source of blood 5-HT and of urinary 5-HIAA. The results obtained in gastro-enterectomized rats minimize the role of mast cells in the general metabolism of 5-HT in rats, since, in spite of repeated parenteral administration of L-tryptophan, these rats excreted $< 10\%$ of the normal 5-HIAA.

5. The biosynthesis of 5-HT in the brain seems largely independent of that in the gastro-intestinal mucosa. The significance of the decrease in brain 5-HT after removal of the large intestine is obscure. The maintenance of a normal 5-HT content in the brain during the 24–72 hr period following total gastro-enterectomy supports the hypothesis that neural 5-HT is of local origin.

This work was supported by a grant from the Rockefeller Foundation, New York.

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