OBSERVATIONS ON THE RELEASE AND TURNOVER RATE OF 5-HYDROXYTRYPTAMINE IN THE GASTROINTESTINAL TRACT

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Three groups of experiments were carried out on man, dogs and rabbits to strengthen the theory of the intestinal origin of blood 5-hydroxytryptamine (5-HT) and urinary 5-hydroxyindoleacetic acid (5-HIAA), and to contribute to the solution of the problem of the turnover rate of 5-HT in the gastrointestinal mucosa. Serum obtained from blood taken by catheterisation from the hepatic veins contained, in man and dogs, more 5-HT than serum obtained from vena cava blood. Moreover, dogs deprived operatively of the entire gastrointestinal tract presented a sharp drop in the urinary 5-HIAA excretion. It was tentatively calculated, from the data gathered in this study, that half-life of gastrointestinal 5-HT is approximately 6 to 8 hours in dogs, and 7 to 12 hours in man.

THE enterochromaffin cells of the gastrointestinal mucosa have been considered for 20 years to be the main site of production and storage of 5-hydroxytryptamine (5-HT, enteramine) in vertebrates[1,2].

Toh[3] was the first to produce direct evidence that blood 5-HT originates from the intestines. He found that in dogs portal blood contained nearly three times more 5-HT than arterial blood, and that there was a spontaneous release of 5-HT from the perfused dog stomach, the output being 0·05 to 0·4 μg. per minute. Bertaccini[4], in this Institute, demonstrated that removal of the large intestine produced in the rat a decrease in the daily urinary excretion of 5-HIAA, that is, a decrease in the daily production and metabolism of 5-HT. Similar results were obtained, quite recently, by Rosenberg and others[5].

According to Erspamer[6] the 5-HT in the rat gastrointestinal tract is completely renewed, in about 8 to 9 hours; according to Udenfriend and Weissbach[7], who employed radioactive precursor amino acids, the biologic half-life of 5-HT in the gastrointestinal mucosa of the rabbit is 11 to 17 hours. This means that the gastrointestinal mucosa of the rat and the rabbit would be capable of synthesising an amount of 5-HT corresponding to that contained in it, in 8 to 9 hours and 22 to 34 hours, respectively.

The three groups of experiments, of which an account is given in this paper, were designed to provide further evidence of the production and release of blood 5-HT from the gastrointestinal tract, and further information about the turnover-rate of gastrointestinal 5-HT.

EXPERIMENTAL AND RESULTS

Differences in the 5-HT Content of Serum Obtained from Vena Cava Blood and of Serum Obtained from Hepatic Veins Blood

Blood samples were taken, by catheterisation, from the hepatic veins and from the vena cava, below the inflow of the renal veins, in six

unanaesthetised patients suffering from heart disease and in six unanaesthetised normal dogs. After standing for 4 to 5 hours at room temperature and then overnight in the refrigerator at 3°, the blood samples were centrifuged and the serum treated with 4 volumes of acetone. The 5-HT content of the filtrate was estimated on the atropinised oestrus uterus of the rat[1,8].

During the week preceding the withdrawal of blood, two successive collections of urine were taken, over 24-hour periods, the first from untreated human beings and dogs, the second from subjects treated subcutaneously with 6 mg. (dogs) and 20 mg. (man) of 5-HT creatinine sulphate. In all the urine samples, 5-HIAA was estimated quantitatively by the method of Macfarlane and others[9], to establish the normal urinary output of 5-HIAA in the examined subjects, and the recovery, as excess urinary 5-HIAA, of the injected 5-HT (1 mg. 5-HIAA is equivalent to 0·92 mg. 5-HT).

The daily urinary excretion of 5-HIAA is shown in Tables I and II. The average recovery of exogenous 5-HT, as urinary 5-HIAA, was found to be for man 32 per cent (range 22 to 45 per cent), for dogs 27 per cent (range 20 to 37 per cent). Thus, the ratio of administered 5-HT to recovered 5-HT was 3·1 for men, and 3·7 for dogs. The values found for man agree very well with those reported by Ferrari and Castelli[10].

The daily release and metabolism of 5-HT was tentatively calculated:

(1) from the difference in the 5-HT content of the serum from hepatic venous blood and the serum from vena cava blood, multiplied by the millilitres of serum which pass through the liver during a 24-hour period. Hepatic blood flow is estimated to be approximately 2200 litres/24 hours (= approximately 1100 litres serum/24 hours) for an adult man, and 53 litres/kg./24 hours (= approximately 27 litres serum/kg./24 hours) for the dog[11].

(2) from the normal urinary output of 5-HIAA multiplied by 3·1 in man and 3·7 in dogs. In this calculation it is, of course, arbitrarily assumed that equivalent amounts of both exogenous and endogenous 5-HT give origin to identical amounts of urinary 5-HIAA.

TABLE I

DIFFERENCES IN THE 5-HT CONTENT OF SERA OF DIFFERENT ORIGIN AND DAILY URINARY EXCRETION OF 5-HIAA IN HUMAN PATIENTS. TENTATIVE CALCULATION OF THE DAILY RELEASE AND METABOLISM OF 5-HT

Patient	5-HT content in hepatic (H) and cava (C) serum (μg./ml.)	Excess 5-HT in hepatic serum (μg./ml.)	Calculated daily release of 5-HT (mg.)	Daily urinary excretion of 5-HIAA (mg.)	Calculated daily metabolism of 5-HT (mg.)
D.C.	H 0·086 C 0·06	0·026	28·6	1·13	3·50
B.R.	H 0·19 C 0·158	0·032	35·2	2·60	7·96
N.P.	H 0·13 C 0·104	0·026	28·6	2·26	7·00
D.G.	H 0·083 C 0·078	0·005	5·5	1·85	5·63
P.L.	H 0·11 C 0·095	0·015	16·5	2·90	9·00
A.G.	H 0·098 C 0·078	0·02	22·0	3·50	10·85
	Mean =	0·021	22·7	2·37	7·32

It appears from Tables I and II that in 11 of 12 experiments the blood serum from hepatic veins contained more 5-HT than that from vena cava. The difference ranged from 0·005 to 0·032 μg./ml. for man and from 0·03 to 0·075 μg./ml. for dogs. Toh's[3] data on the release of 5-HT from the dog's gastrointestinal tract were thus confirmed, but the rate of release was found to be considerably less than that stated by this investigator.

TABLE II

DIFFERENCES IN THE 5-HT CONTENT OF SERA OF DIFFERENT ORIGIN AND DAILY URINARY EXCRETION OF 5-HIAA IN DOGS. TENTATIVE CALCULATION OF THE DAILY RELEASE AND METABOLISM OF 5-HT

Dog	5-HT content in hepatic (H) and cava (C) serum (μg./ml.)	Excess 5-HT in hepatic serum (μg./ml.)	Calculated daily release of 5-HT (mg./10 kg.)	Daily urinary excretion of 5-HIAA (mg./10 kg.)	Calculated daily metabolism of 5-HT (mg./10 kg.)
1	H 0·45 C 0·42	0·03	7·1	0·67	2·5
2	H 0·26 C 0·20	0·06	14·2	0·86	3·2
3	H 0·215 C 0·237	—	—	0·64	2·4
4	H 0·494 C 0·451	0·043	11·6	1·10	4·1
5	H 0·286 C 0·25	0·036	9·7	1·23	4·6
6	H 0·645 C 0·57	0·075	20·2	0·40	1·5
	Mean =	0·05	12·6	0·82	3·05

The daily production of 5-HT appeared to be generally greater, approximately three- to four-fold, if calculated from the difference in the 5-HT content between blood returning from the intestines and vena cava blood, than if calculated from the urinary output of 5-HIAA. We are inclined to consider the values given by the latter method to be more precise. In the first calculation, indeed, the data concerning the 5-HT content, were necessarily obtained by a biological method which is probably less accurate than a chemical method. Moreover, in this calculation it was assumed, presumably incorrectly[12], that release of 5-HT from the enterochromaffin cells is continuous and uniform.

5-HT Content in Blood Plasma of Rabbits Pretreated with Reserpine

To obtain additional and, if possible, more accurate data on the 5-HT release from the gastrointestinal mucosa, a few experiments were made on reserpine-pretreated animals.

Reserpine is known to deplete the 5-HT depots in the organism. Particularly sensitive to the drug are the blood platelets, which not only lose nearly the whole of their 5-HT but become also incapable of absorbing 5-HT from the surrounding medium[13]. Reserpine, on the other hand, does not seem capable of interfering in the biosynthesis of 5-HT[14]. It seems, therefore, conceivable that in animals given reserpine the 5-HT normally released from the gastrointestinal mucosa would appear free in the plasma and that, as a consequence, plasma from blood of intestinal origin would contain more 5-HT than plasma from blood returning from other areas.

Experiments were carried out on three rabbits, weighing 3 to 5 kg. After a blood collection from the ear marginal vein (control serum), the animals were given intravenously 0·2 mg./kg. of reserpine. Forty-eight hours later, a second blood sample was taken from the ear vein (reserpine serum); then the animals were given heparin intravenously and soon after under a light ether anaesthesia, blood samples were withdrawn through polythene catheters from the jugular vein and from a mesenteric vein into siliconised centrifuge tubes. Plasma was separated by centrifugation at 3000 rev./min. for 20 minutes, then treated with 4 volumes of acetone. 5-HT was estimated in the filtrate. Results are shown in Table III.

TABLE III

THE 5-HT CONTENT OF PLASMA OBTAINED FROM MESENTERIC VENOUS BLOOD AND FROM JUGULAR BLOOD OF RABBITS PRETREATED WITH RESERPINE

	5-HT content (μg./ml.) in			
	Control serum	Reserpine serum	Reserpine mesenteric plasma	Reserpine jugular plasma
Rabbit 1	3·1	0·025	<0·02	<0·02
,, 2	2·2	0·2	<0·02	<0·02
,, 3	4·5	0·12	<0·02	<0·02

It may be seen that under our experimental conditions no difference could be found in the 5-HT content of plasma from mesenteric vein blood and plasma from jugular vein blood. It follows that in the rabbit the release of 5-HT by the gastrointestinal mucosa is less than 0·02 μg./ml. The rat uterus preparation is unsuitable for an accurate estimation of lower concentrations of 5-HT. We are now repeating our experiments in rabbits and dogs given reserpine, using the more sensitive Vane's method[15].

Serum 5-HT and Urinary 5-HIAA Following Removal of the Gastrointestinal Tract in Dogs

Two dogs were subjected, under pentobarbitone anaesthesia, to removal of the entire gastrointestinal tract, the spleen and the pancreas. The operation was preceded by the withdrawal of a sample of arterial blood and an estimation of 5-HIAA in urine collected over a 24-hour period. After the operation the dogs were given antibiotics and sufficient amounts of physiological saline, in part intravenously and in part subcutaneously. One dog died after 48 hours, the second after 25 hours. During the survival period urine was carefully collected and blood samples drawn from the femoral artery after 20 and 40 hours. The results are set down in Table IV. This Table shows two interesting facts.

Removal of the gastrointestinal tract, that is, of the enterochromaffin cell system, does not apparently modify, during the brief survival period, the 5-HT content of serum. Without excluding that a real decrease in the 5-HT content of blood may be in part masked by a concomitant *inspissatio sanguinis*, we consider that the observed fact is strongly indicative of the tenacity with which platelets retain their 5-HT or at least a

remarkable aliquot of it. This agrees well with the calculated half-life of platelet 5-HT (33 to 48 hours[7]).

The 5-HIAA content of urine declines rapidly after the operation. The urine of the first 24 hours contains about 20 to 25 per cent of the 5-HIAA in normal urine; later on, this metabolite seems to disappear completely.

TABLE IV

SERUM 5-HT LEVELS AND URINARY 5-HIAA EXCRETION IN DOGS AFTER REMOVAL OF THE GASTROINTESTINAL TRACT

	Serum 5-HT μg./ml.	Urine volume ml./24 hours	Urinary 5-HIAA μg./24 hours
Dog 1 (12 kg.)—			
Before operation	0·42	756	674
0–24 hours after operation ..	0·47	230	170
24–48 hours after operation ..	0·30	400	? (<40)
Dog 2 (14·5 kg.)—			
Before operation	0·20	1013	860
0–25 hours after operation ..	0·23	200	180

We are perfectly aware of the extreme caution with which these results should be interpreted. These are, however, in good accordance with those obtained by other research workers[4,5] in very extensive studies.

DISCUSSION

The experiments described in this paper support the theory that blood 5-HT originates from the gastrointestinal mucosa. Moreover, this mucosa seems to be, on the basis of the available data, the only source of all the extracerebral 5-HT and, as a consequence, of all the urinary 5-HIAA. A possible exception is found, among the mammals, in some rodents (rats and mice) in which the mast cells also may in theory contribute to the biosynthesis of blood 5-HT and urinary 5-HIAA. Experiments are in progress in this laboratory with the purpose of throwing some light on the problem of the part played by the rat mast cells in the production of the 5-HT found in blood and the 5-HIAA found in urine.

The data here presented may also help in the elucidation of the problem of the turnover rate of gastrointestinal 5-HT in mammals, more precisely in that of the man and the dog.

If we accept that, for a dog weighing 10 kg., the 0·82 mg. of 5-HIAA excreted over a 24-hour period, originates from 3 mg. of 5-HT, then we ought to conclude that a quantity of 5-HT corresponding to that contained in the entire gastrointestinal tract (approximately 1·5 to 2 mg.) is synthesised every 12 to 16 hours. The half-life of gastrointestinal 5-HT would be approximately 6 to 8 hours.

For man, assuming that the 1500 g. of gastrointestinal tract contains an average of 3 to 5 μg. of 5-HT per g. of fresh tissue[8], the total content of the gastrointestinal tract would be 4·5 to 7·5 mg. 5-HT. If the figure for the daily metabolism of 5-HT, calculated from the urinary 5-HIAA,

is reasonably accurate (7·3 mg.), then a quantity of 5-HT corresponding to that contained in the entire gastrointestinal tract would be synthesised every 14 to 24 hours, that is, the half-life of gastrointestinal 5-HT would be approximately 7 to 12 hours.

It may be seen that the above values agree fairly well with those obtained by Udenfriend and Weissbach[7], who used radioactive tryptophan and 5-hydroxytryptophan and found that in the rabbit the half-life of intestinal 5-HT was 11 hours.

It should be kept in mind, however, that there is increasing evidence demonstrating that the biosynthetic possibilities of the enterochromaffin cells are far from being completely exhausted under normal conditions. Some experimental results, for example, seem to indicate that the turnover rate of gastrointestinal 5-HT may be conspicuously increased when excess dietary tryptophan is offered to the enterochromaffin cells. In fact, Lauer and others[16] and Kopin[17] found that human subjects doubled their urinary excretion of 5-HIAA in the 6-hour period after oral administration of 5 g. of L-tryptophan.

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