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5-HYDROXYINDOLE METABOLISM IN THE BRAIN AFTER HEPATECTOMY*

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Abstract—The concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) increased in the brain of dogs and rats after complete hepatectomy, the increases of 5-HIAA being greater than those of 5-HT. The increase in concentration of both 5-hydroxyindoles was much smaller than that of the precursor, tryptophan (Try). The demonstration of radioactive 5-HT and 5-HIAA in the brain of normal and hepatectomized rats after the intradiencephalic injection of L-Try-3-^{14}C indicated that hydroxylation of Try occurred in brain *in vivo*. However, the intravenous injection of Try (33 mg/kg) resulted in definite increases in the concentration of 5-hydroxyindoles in the brain of normal rats and only marginal increases in the brain of hepatectomized rats. After intravenous injection of D,L-5-hydroxytryptophan, conversion to 5-HT was essentially similar in normal dogs and in dogs in coma after complete hepatectomy.

CHANGES in concentration in brain of 5-hydroxytryptamine (5-HT, serotonin), a metabolite of tryptophan (Try), are known to produce neurologic symptoms. Borges and co-workers[1] in 1959 suggested that, if hydroxylation of Try occurred exclusively in the liver, a deficiency of 5-HT might develop in the brain of patients with severe liver disease and contribute to the symptoms of hepatic coma. They obtained more normal electroencephalograms in comatose patients after the administration of 5-hydroxytryptophan (5-HTP). However, slight increases in the concentration of 5-HT in brain were found in hepatectomized dogs in coma[2] and large increases in both 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were found in rats after hepatectomy.[3] Gal and co-workers[4, 5] have shown clearly that hydroxylation and decarboxylation of intracerebrally injected L-Try-^{14}C occurred, with formation of 5-HT-^{14}C in brains of rats and pigeons. They concluded, however, that synthesis of 5-HTP in brain could not account for all the 5-HT in this organ and, thus, that brain must rely on other tissues for much of this amino acid. Weber and Horita[6] showed that similar amounts of 5-HT were formed from Try in brain of totally eviscerated rats and of control rats (no operation). Thus, brain did not depend on the liver, stomach, intestine, spleen, or kidneys for 5-HTP.

In the present investigations we have explored further the role of 5-HT in hepatic coma; studies have been made of 5-hydroxyindoles and of Try in the brain of

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hepatectomized dogs in coma and of the effects of injections of 5-HTP and dihydroxyphenylalanine (dopa) on 5-hydroxyindole concentrations. We have also investigated the alterations in 5-hydroxyindole and in Try metabolism that develop in rat brain after hepatectomy by evisceration. Preliminary reports of this work have been presented elsewhere.[2, 3, 7]

METHODS

Experimental procedures in dogs. Dogs weighing 7–15 kg were used in these studies. Total hepatectomy was done in some of them by the two-stage technique of Grindlay and Mann[8] or by the one-stage technique of Starzl and co-workers.[9] The dogs were catheterized, and urine was collected continuously in ice-cold flasks containing 5 ml of glacial acetic acid. When the dogs recovered from ether anesthesia, they were maintained by continuous infusion of glucose at the rate of 25 mg/kg/hr. Blood samples were taken before and at intervals after hepatectomy. When the dogs manifested symptoms of hepatic coma, they were anesthetized with sodium pentobarbital (25 mg/kg), the aorta was sectioned, and the brain was removed rapidly. The brain was dissected into five or six regions, as described previously,[10] and frozen immediately in powdered Dry Ice. Similar studies were done on control dogs (no operation).

In some dogs an Eck fistula was created by end-to-end anastomosis of the portal vein and inferior vena cava. Four to six months later, the brains were removed from these dogs when they were in coma after the administration of a meat meal.

In some experiments, D,L-5-HTP dissolved in physiologic saline (pH 7·2) was administered to normal dogs by a continuous infusion during a 4-hr interval (dose: 54 mg/kg) and to comatose hepatectomized dogs (dose, mg/kg: 25, 40, 54, respectively, to three dogs) over periods of 3 to 4 hr. Two other hepatectomized dogs in coma each received a single i.v. injection of this substance (2 mg/kg). Another three comatose hepatectomized dogs received dopa in doses of 100, 115, or 150 mg/kg, respectively, by continuous infusion over 4 hr. Thirty minutes after the end of the infusion, the dogs were killed and the brains removed.

Experimental procedures in rats. Rats weighing 230–300 g were used in these studies. Hepatectomy was done, under ether anesthesia, by the two-stage evisceration described previously.[11] Cystostomy was done to allow continuous collection of urine. The rats were maintained, without anesthesia, by continuous infusion of glucose (15–25 mg/100 g/hr) dissolved in physiologic saline (1·25 ml/hr). At intervals of 6–24 hr after hepatectomy, the rats were anesthetized with ether for 1·5 min, blood was taken by cardiac puncture, and the brain, heart, and a sample of skeletal muscle were removed and frozen. Similar studies were made of control rats and of rats after laparotomy (the latter received a continuous infusion of saline).

In other experiments, Try (33 mg/kg body weight) was injected into the tail vein over a period of 2 min to normal rats and to rats 20 hr after hepatectomy. At intervals after the injection of Try, blood and tissues were removed.

In some of the rats, at the time of hepatectomy a small hole (1 mm from the sutura sagittalis and 1 mm from the sutura coronalis) was drilled almost through the skull with a dental drill. Control rats were prepared in a similar manner. Twenty hr later, with the rats under light ether anesthesia, a hypodermic needle 8 mm long was inserted through the drilled hole at angles of 10° to the vertical and frontal planes.

Injections of radioactive Try [2 μc (15·6 μg) of L-Try-3-^{14}C, sp. act. 26·2 mc/m-mole (Nuclear-Chicago Corp.) in 20 μl] were then made directly into the diencephalic zone. After withdrawal of the needle, the puncture was swabbed, the swab was immersed in water, and aliquots of the water wash were counted for radioactivity. If more than 12 per cent of the injected Try-^{14}C was found on the swab, that experiment was excluded from the series. At 10, 30, or 60 min after injection the rats were anesthetized with ether and the brains removed.

Chemical procedures. Fluorometric methods were used to measure concentrations of total 5-hydroxyindoles,[12] 5-HIAA,[13] and 5-HT[14] in tissues. The concentration of 5-hydroxyindoles in blood was measured by the method of Udenfriend and co-workers.[15] The concentration of 5-HIAA in urine was determined by the method of Macfarlane and co-workers.[16] The concentrations of Try in tissues and in plasma were determined by the method of Hess and Udenfriend.[17] Because the fluorometric method used to measure Try does not distinguish between Try and tryptamine, in some experiments the amine was extracted from tissue homogenates and determined separately.[17] Tyrosine (Tyr) concentrations were determined in trichloroacetic acid (TCA) filtrates by the method of Waalkes and Udenfriend.[18]

Separation of metabolites of L-*Try-*^{14}*C and measurement of radioactivity.* Brains were homogenized in (1) 80% alcohol containing 0·2% ascorbic acid or (2) 6 volumes of 0·1 N HCl with trichloroacetic acid added to make a 6% solution. Radioactivity in the homogenates and in supernatant fractions was measured, in Kinard's[19] scintillation mixture containing 4% Cab-O-Sil, in a liquid scintillation counter (Packard) with an efficiency of 63 per cent. The radioactivity in proteins or proteolipids was calculated by the difference between these determinations. Corrections were made for quenching by the addition of internal standard. Radioactivity is reported as counts per minute (cpm).

The alcoholic extracts of brains were transferred to Amberlite CG-50 columns, and the radioactive metabolites of L-Try-^{14}C were separated by the method of Davis and co-workers.[20] 5-HT was eluted from the column in 4 N acetic acid, with a mean recovery of 85% (range, 82–88%). The effluent from the column was concentrated to about 0·5 ml, mixed with 2 ml water, 5 ml 95% ethanol, and 10 ml chloroform, and centrifuged. The aqueous layer was removed and the organic layer washed twice with 2 ml water. The combined aqueous layers were taken to dryness *in vacuo*, and the dried residue was dissolved in water.

Paper chromatography in isopropyl alcohol:ammonia:water (20:1:2) or in butanol:acetic acid:water (4:1:5) and high-voltage electrophoresis showed that the only radioactive compounds present were 5-HT in the eluate from the column and 5-HIAA and Try in the effluent.

Electrophoretic separation was done in a high-voltage paper electrophorator on sheets of Whatman 3MM paper (6 in. × 4 ft). Aliquots of unknown solutions were applied at each side and 1 in. apart from a standard mixture of indoles. After electrophoresis for 1·5 hr at 5000 V at pH 1·3 in formic acid buffer (1·3 l. of 16% formic acid, 190 ml of glacial acetic acid, and 10·6 g of cadmium acetate made up to 10 l. with water), the papers were dried for 30 min at 80° and cut into three strips; the strip carrying the standards was sprayed with Ehrlich's reagent. Indolic compounds travelled the following distances from the origin: tryptamine, 43 cm; 5-HT, 36 cm; Try, 29 cm; 5-HTP, 25 cm; and 5-HIAA, 6 cm. The two side strips containing the sample were

cut into 1-cm pieces which were immersed in Liquifluor for measurement of radioactivity in the scintillation counter.

The recoveries through the extraction and electrophoresis were 58–65% for Try and 60–68% for 5-HIAA. Because of difficulties in drying the eluates of the column completely, an internal standard of glycine-^{14}C was added before concentration and electrophoresis, and corrections were made for variation in volume and losses due to self-absorption in the paper. The glycine-^{14}C traveled 50 cm from the origin and thus was well separated from the indoles.

RESULTS

Try concentrations after hepatectomy. The concentration of Try in plasma and tissues increased two- and fourfold in plasma and tissues of dogs after hepatectomy (Table 1). The increases were greater in brain than in plasma, heart, or skeletal muscle,

TABLE 1. EFFECT OF HEPATECTOMY ON TRYPTOPHAN AND TYROSINE IN PLASMA AND TISSUES OF DOGS*

	Normal		20–24 hr after hepatectomy	
	Tryptophan	Tyrosine	Tryptophan	Tyrosine
Plasma	10·5 ± 1·0 (11)	9·0 ± 0·6 (9)	21·3 ± 1·7 (6)	51·3 ± 5·4 (6)
Heart	6·3 ± 0·3 (7)	16·5 ± 0·6 (7)	16·1 ± 1·5 (6)	59·0 ± 4·7 (6)
Skeletal muscle	5·5 ± 0·6 (7)	17·3 ± 1·2 (7)	13·2 ± 1·1 (6)	60·3 ± 4·4 (6)
Cerebrum	5·7 ± 0·3 (6)	16·5 ± 1·1 (6)	24·3 ± 1·5 (5)	76·6 ± 3·9 (5)
Brain stem	6·2 ± 0·2 (8)	13·9 ± 1·1 (8)	28·8 ± 2·1 (7)	82·3 ± 9·4 (7)
Cerebellum	7·2 ± 0·3 (7)	18·9 ± 1·4 (7)	32·6 ± 4·1 (6)	106·8 ± 8·4 (6)
Caudate nucleus	6·5 ± 0·7 (7)	14·9 ± 1·0 (7)	22·5 ± 2·4 (6)	84·4 ± 11·7 (6)
Hypothalamus	6·3 ± 0·3 (7)	21·5 ± 1·9 (6)	24·1 ± 1·6 (6)	76·0 ± 9·4 (5)
Hippocampus	6·5 ± 0·4 (7)	17·7 ± 1·4 (7)	26·5 ± 1·3 (6)	88·4 ± 8·5 (6)

* Data shown, as μg/g or ml for plasma, are means ± S.E. The number of determinations is in parentheses.

and the brain-to-plasma ratio was higher in hepatectomized than in normal dogs. Even larger increases in tyrosine concentrations were found after hepatectomy.

In the rat also, two- and fourfold increases in Try concentrations in plasma and in tissues occurred after hepatectomy (Table 2). In contrast to results in the dog (Table 1), concentrations of both Try and Tyr were less in brain than in plasma, heart, or skeletal muscle 24 hr after hepatectomy. The concentration of Tyr increased more rapidly than that of Try.

5-Hydroxyindole concentration after hepatectomy. In nine hepatectomized dogs the concentration of 5-HT in blood decreased to 24% of the preoperative level; Fig. 1 shows this decrease in four of the nine dogs during the 24 hr after hepatectomy. A similar decrease in platelet count was found in the one dog in which measurements were made. It is well known that a reduction in circulating platelets may occur after a major surgical procedure.

The average excretion of 5-HIAA in 64 samples of 24-hr urine from seven normal dogs was 63 ± 2·1 (S.E.) μg/hr (1·5 ± 0·1 mg/24 hr). The urinary output of 5-HIAA

by dogs after hepatectomy was similar (Fig. 1). Small increases of questionable significance were found in concentration of 5-HT in brains of hepatectomized dogs (Fig. 2). The concentrations of 5-HT in brain of comatose dogs with an Eck fistula were normal in the brain stem (0·71, 0·67, and 0·68 μg/g) and in the caudate nucleus

TABLE 2. EFFECT OF HEPATECTOMY ON TRYPTOPHAN AND TYROSINE IN PLASMA AND TISSUES OF RATS*

	Normal	After hepatectomy	
		16 hr	24 hr
		Tryptophan	
Plasma	12·1 ± 0·6 (10)	25·6 ± 2·1 (6)	31·9 ± 2·0 (6)
Heart	10·9 ± 0·4 (8)	27·7 ± 0·8 (5)	32·5 ± 2·5 (6)
Skeletal muscle	8·2 ± 0·4 (8)	24·5 ± 1·8 (6)	39·6 ± 3·1 (6)
Brain	6·6 ± 0·3 (10)	17·3 ± 1·1 (6)	22·5 ± 1·6 (8)
		Tyrosine	
Plasma	15·5 ± 1·0 (10)	91·9 ± 4·9 (6)	138·2 ± 4·0 (6)
Heart	20·5 ± 0·5 (8)	88·0 ± 5·6 (6)	133·0 ± 6·3 (6)
Skeletal muscle	25·9 ± 0·7 (8)	96·5 ± 5·9 (6)	126·6 ± 3·5 (6)
Brain	19·3 ± 0·9 (10)	54·8 ± 1·4 (6)	89·1 ± 5·0 (6)

* Data shown, as μg/g or ml for plasma, are means ± S.E. The number of determinations is in parentheses.

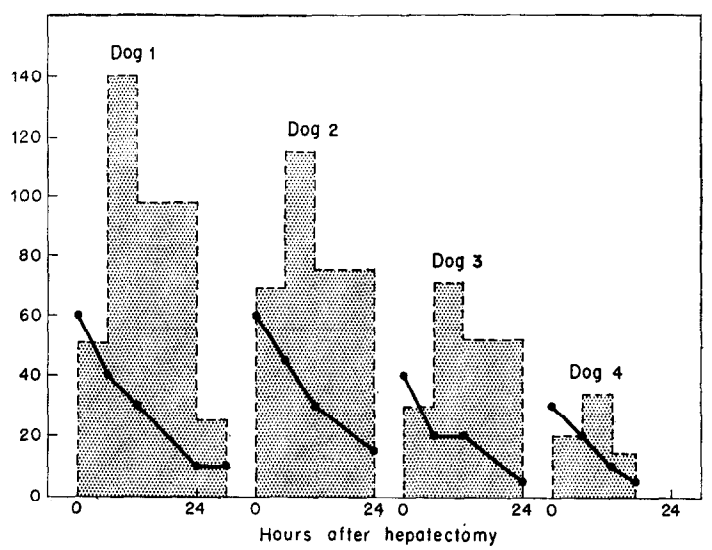

FIG. 1. Concentration of 5-hydroxyindoles in blood and urine of four dogs at different times after hepatectomy. The solid lines show 5-hydroxytryptamine in blood as micrograms per 100 ml. The stippled bars show 5-hydroxyindoleacetic acid in urine as micrograms per hour.

(0·51, 0·44, and 0·63 μg/g) and were in the low-normal range in the hypothalamus (1·05, 0·92, and 0·86 μg/g). In the hepatectomized dogs there were also progressive increases in the concentration of 5-HIAA in all regions of the brain, to amounts about twice normal after 24 hr (Table 3). No tryptamine was detected in brain of normal or hepatectomized dogs.

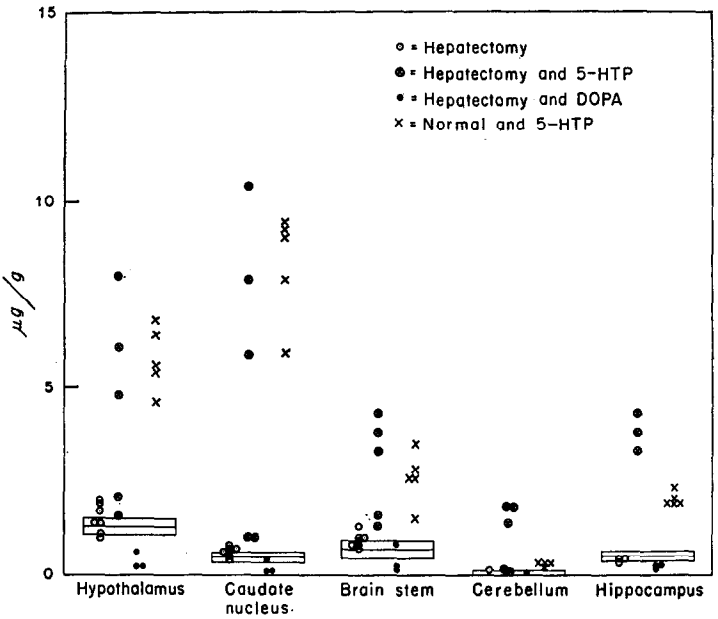

FIG. 2. 5-Hydroxytryptamine (5-HT) in canine brain. Horizontal lines show mean concentrations of 5-HT in brains of eight control dogs, with one standard deviation on each side. 5-Hydroxytryptophan (5-HTP) was injected into hepatectomized animals in the following concentrations: 2 mg/kg to two dogs and 25, 40, and 54 mg/kg, respectively, to three dogs. The concentrations in brain were proportional to the amounts of 5-HTP injected. 5-HTP (54 mg/kg) was injected into five normal dogs. Dopa was injected into three hepatectomized dogs (100, 115, and 150 mg/kg, respectively). The lowest concentration of 5-HT was found in the dog receiving the highest dose of dopa.

TABLE 3. 5-HYDROXYINDOLEACETIC ACID IN CANINE BRAIN*

Region of brain	5-Hydroxyindoleacetic acid (μg/g)			
		After hepatectomy		
	Normal (8)	6 hr	18 hr	24 hr†
Hypothalamus	1·33 ± 0·09	1·32, 1·83	1·76	2·52, 2·55, 2·67
Brain stem	1·24 ± 0·11	1·37, 2·08	2·98	2·99 ± 0·33 (6)† ($P < 0·001$)
Caudate nucleus	0·52 ± 0·04	0·64, 0·64	0·62	0·89, 0·90, 0·90
Hippocampus	0·44 ± 0·04	0·49, 0·60	0·56	0·74 ± 0·12 (4) ($P < 0·01$)
Cerebrum	0·15 ± 0·01	0·26, 0·35	0·17	0·29 ± 0·02 (4) ($P < 0·001$)
Cerebellum	0·07 ± 0·01	0·08, 0·09	0·13	0·18 ± 0·03 (4) ($P < 0·001$)

* Data shown as means ± S.E. or as single values. The number of determinations is in parentheses except where single values are given.

† P values are for differences from normal.

In the rat, the concentration of 5-hydroxyindoles decreased in blood and heart after hepatectomy or laparotomy (Table 4). The urinary output of 5-HIAA decreased after hepatectomy but not after laparotomy. Concentrations of both 5-HT and 5-HIAA increased in brain of rats after hepatectomy. Little if any tryptamine was detected in brain of normal or hepatectomized rats.

TABLE 4. 5-HYDROXYINDOLES AND TRYPTAMINE IN THE RAT AFTER HEPATECTOMY*

	Normal	After hepatectomy* 12 hr	After hepatectomy* 24 hr	24 hr after laparotomy†
5-Hydroxyindoles in blood (μg/ml)	1·62 ± 0·15 (11)	1·33 ± 0·10 (9)	1·20 ± 0·15 (10) ($P < 0·05$)	1·09 ± 0·07 (8) ($P < 0·05$)
5-HT in heart (μg/g)	0·81 ± 0·03 (10)	0·46 ± 0·05 (7) ($P < 0·001$)	0·65 ± 0·04 (10) ($P = 0·01$)	0·43 ± 0·08 (8) ($P = 0·001$)
5-HT in brain (μg/g)	0·55 ± 0·02 (12)	0·96 ± 0·03 (5) ($P < 0·001$)	0·96 ± 0·06 (12) ($P < 0·001$)	0·61 ± 0·03 (6)
5-HIAA in brain (μg/g)	0·41 ± 0·01 (12)	0·92 ± 0·06 (7) ($P < 0·001$)	1·30 ± 0·12 (5) ($P < 0·001$)	0·43 ± 0·01 (6)
Tryptamine in brain (μg/g)	0–0·12 (6)	0–12 hr	0–0·07 (4) 12–24 hr	0–24 hr
5-HIAA in urine (μg/hr)	4·4 ± 0·2	1·4 ± 0·3 (9) ($P < 0·001$)	1·0 ± 0·3 (8) ($P < 0·001$)	4·3 ± 0·02 (12)

* Data are means ± S.E. The number of determinations is in parentheses.
† P values are for differences from normal.

Administration of D,L-*5-HTP to dogs.* 5-HTP was infused into five comatose dogs on which electroencephalograms (EEG) were made. When 2 mg of 5-HTP/kg (a dose approximating that causing improvement in patients in hepatic coma[1]) was infused into two comatose dogs, there were no appreciable increases in 5-HT concentration in the brain (Fig. 2) and no change in EEG. A range of doses of 5-HTP was tested; 24, 40, or 54 mg/kg produced substantial increases in the concentration of 5-HT in brain in three comatose dogs. The 5-HTP was well tolerated by the comatose dogs, but in none was there improvement in the EEG or reversal of the symptoms of coma.

Confirming earlier reports,[21] the administration of 5-HTP at 54 mg/kg to normal dogs brought about increases in the concentration of 5-HT in all areas of the brain. Higher concentrations were found in brains of some of the hepatectomized dogs than in brains of normal dogs. This could be due to the larger amounts of 5-HTP available to the brain when metabolism by the liver was eliminated. 5-HTP was less well tolerated by the normal dogs and produced the symptoms already described.[21]

Dopa administered in concentrations of 100, 115, or 150 mg/kg to three comatose dogs caused substantial decreases in the concentrations of 5-HT in the brain. Infusion of dopa did not bring about clinical or EEG improvement.

In confirmation of earlier studies,[10] increases in concentration of 5-HT were found in the mucosa of the pyloric region of the gastrointestinal tract of dogs after hepatectomy. The administration of 5-HTP did not induce further increases in this region nor did it cause increases in the duodenum, jejunum, or ileum. Only minimal increases

were found in all the regions examined after the administration of 5-HTP at 54 mg/kg to normal dogs, possibly because of the slower turnover of 5-HT in gastrointestinal mucosa than in brain.[22] The administration of dopa resulted in slightly decreased concentrations of 5-HT in gastrointestinal mucosa.

Administration of Try to rats. When Try was administered i.v. to normal rats in doses of 33 mg/kg, there was a rapid uptake in liver and heart, with maximal concentrations in 2·5 min (Fig. 3). Then the concentrations of Try in plasma, liver, and heart

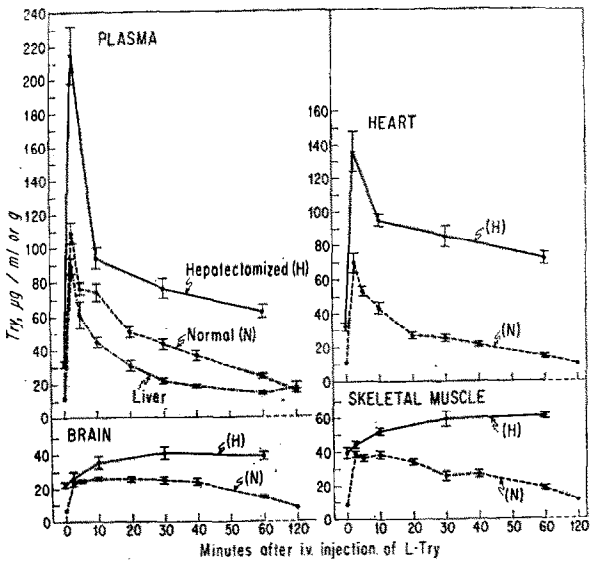

Fig. 3. Fate of injected tryptophan (Try) in rats. Normal (N) or hepatectomized (H) rats were injected i.v. with Try (33 mg/kg in 2 ml) over a period of 2 min. At different times after the end of injection, rats were killed and tissues and blood were removed for Try determinations. The values shown are means ± S.E. of 4 to 12 determinations (usually 8 to 10). Where S.E. is not shown, it was too small to be represented graphically.

decreased rapidly. The peak concentrations were smaller and the rates of disappearance were much slower in skeletal muscle and brain than in heart and liver. Higher concentrations of Try were found in plasma and heart of hepatectomized rats than in normal rats throughout the 60 min after the injection of Try. The concentration of Try increased in skeletal muscle for 30 min and then remained constant for the next 30 min. In normal rats there was an increase of 17·0 μg/g in Try in the brain at 2·5 min after injection (from 6·6 to 23·6 μg) in contrast to 4·2 μg/g (from 22·5 to 26·7 μg) in hepatectomized rats. Thus the net uptake of Try in the brain was less rapid in hepatectomized rats, although the concentration of Try in plasma was greater than in normal rats (215 μg/ml compared to 109 μg/ml).

In normal rats, administration of Try (33 mg/kg) produced significant increases in the concentration of 5-HT, which persisted for the 60 min of the experiment (Table 5). However, these increases were modest compared to those of the precursor, Try (Fig. 3). Even smaller but significant increases were found in the concentrations of 5-HIAA in the brain: from 0·44 to 0·66 μg/g. In hepatectomized rats the concentration of 5-HT in the brain increased slightly for 30 min after injection of Try. The changes

in concentration of 5-HIAA in brains of hepatectomized rats after the i.v. injection of Try were not statistically significant.

Metabolism of L-*Try*-3-^{14}C *by rats.* The radioactivity in the brain was fairly evenly distributed at 10 min after injection of 2 μc (15·6 μg) of Try-^{14}C into the diencephalic region. If the radioactivity (in cpm/g) of whole brain was considered to be 1, the relative values for the parts were: cerebrum (on the side of injection), 1·02; cerebrum (opposite side), 0·83; cerebellum, 1·27; and brain stem, 1·09.

At 10 min after injection of Try-^{14}C the total radioactivity in the brain of a normal rat was equivalent to 26% of the 2-μc dose (423 $\times$ 10^3 cpm/g, Table 6). The total

TABLE 5. 5-HYDROXYINDOLES IN RAT BRAIN AFTER TRYPTOPHAN INJECTION*

After injection (min)	Normal†		20–22 hr after hepatectomy†	
	5-HT (μg/g)	5-HIAA (μg/g)	5-HT (μg/g)	5-HIAA (μg/g)
Controls	0·62 ± 0·02 (6)	0·44 ± 0·02 (6)	0·92 ± 0·04 (7)	1·43 ± 0·07 (6)
2·5	0·76 ± 0·04 (6) ($P < 0·01$)	0·50 ± 0·02 (6) ($P < 0·05$)	1·18 ± 0·12 (6) ($P < 0·05$)	1·32 ± 0·07 (5)
10	0·83 ± 0·03 (5) ($P < 0·001$)	0·55 ± 0·01 (5) ($P < 0·01$)	1·06 ± 0·05 (6) ($P < 0·05$)	1·31 ± 0·10 (5)
30	0·92 ± 0·03 (6) ($P < 0·001$)	0·55 ± 0·02 (6) ($P < 0·01$)	1·03 ± 0·03 (7) ($P < 0·05$)	1·34 ± 0·09 (5)
60	0·88 ± 0·05 (6) ($P < 0·001$)	0·66 + 0·03 (5) ($P < 0·001$)	1·02 ± 0·07 (7)	1·46 ± 0·16 (6)

* Normal or hepatectomized rats were injected i.v. with Try (33 mg/kg in 2 ml) over a period of 2 min. At different times after the end of the injection the rats were killed and the brains were removed for determination of 5-HT or 5-HIAA. Data are means ± S.E. The number of determinations is in parentheses.

† P values are for differences from controls.

radioactivity in the brain in other rats at 30 min after injection was much less, and at 60 min it amounted to about 7% of the dose. About half the radioactivity in brain at 10 min was in Try, but only 9·5% was in Try at 60 min. The radioactivity in the protein fraction was equal to that of Try at 30 min and was much higher at 60 min. The radioactivity in 5-HT increased from 2·1 $\times$ 10^3 to 8·0 $\times$ 10^3 cpm/g between 10 and 30 min after injection and then decreased. The radioactivity in 5-HIAA was 4·4 cpm/g at 30 min and was about the same at 60 min. Only a very small fraction of the administered Try was converted to 5-HT or 5-HIAA; together, 5-HT and 5-HIAA accounted for 0·74% of the dose at 30 min and 0·54% at 60 min. The sum of the radioactivity in 5-HT, 5-HIAA, and Try after electrophoresis and in the protein fraction amounted to about 70% of the total radioactivity in the brain homogenate. Much of the loss could be due to self-absorption of radioactivity on the filter paper (only 58–65% of the radioactivity was recovered when Try-^{14}C was added to brain extracts before electrophoresis).

When Try-^{14}C was injected into the brain, the radioactivity disappeared much more slowly in hepatectomized rats than in normal rats: twice as much was present at 30 and 60 min. At 10 min after injection 58·1% of the radioactivity was in unchanged Try, and at 60 minutes, 44·3%. The absolute loss, in cpm/g $\times$ 10^3, of Try from brain

TABLE 6. METABOLISM OF L-TRYPTOPHAN-3-^{14}C IN THE RAT BRAIN AFTER INTRADIENCEPHALIC INJECTION*

	Normal rats			Hepatectomized rats†			
Min after injection	10	30	60	10	10	30	60
No. of determinations	1	8	5	1	1	5	5
Total cpm/g brain × 10^3	423	241 ± 16	112 ± 15	492	518	482 ± 60 ($P < 0.001$)	229 ± 38 ($P < 0.01$)
5-HT:							
cpm/g brain × 10^3	2·1	8·0 ± 1·3	4·8 ± 1·0	1·8	1·0	7·6 ± 1·9	5·5 ± 1·3
% of total ^{14}C in brain	0·5	3·2 ± 0·4	4·2 ± 0·5	0·4	0·2	1·5 ± 0·3 ($P < 0.05$)	2·3 ± 0·2 ($P < 0.01$)
5-HIAA:							
cpm/g brain × 10^3		4·4 ± 0·5	4·1 ± 1·7	4·7		7·0 ± 1·9	10·2 ± 3·9
% of total ^{14}C in brain		1·8 ± 0·1	3·6 ± 0·2	1·0		1·3 ± 0·3	4·0 ± 0·9
Proteins and proteolipids:							
cpm/g brain × 10^3		83·6 ± 11·0	65·3 ± 6·2			54·1 ± 7·4	55·0 ± 8·3
% of total ^{14}C in brain		32·0 ± 2·8	58·8 ± 4·9			10·3 ± 0·9 ($P < 0.001$)	25·2 ± 3·5 ($P < 0.001$)
Try:							
cpm/g brain × 10^3	209·0	81·6 ± 10·8	11·6 ± 4·0	285·9	330·8	247·7 ± 47·0 ($P < 0.01$)	101·4 ± 17·2 ($P < 0.001$)
% of total ^{14}C in brain	49·4	32·6 ± 2·8	9·5 ± 2·0	58·1	63·9	49·8 ± 5·3 ($P < 0.01$)	44·3 ± 1·6 ($P < 0.01$)
Calculation of turnover of Try:							
absolute loss of cpm/g × 10^3 between 10 and 60 min	209–12 = 197					309–100 = 209	
disappearance rate‡ (% min)	5·7					2·5	
pool size (μg/g), endogenous + injected Try§	6·6 + 1·2 = 7·8					20 + 1·7 = 21·7	
approximate turnover (μg/g brain/min)	0·44					0·54	

* Data are means ± S.E.
† P values for differences from normal.
‡ Approximate disappearance rates were obtained by drawing lines through points of cpm/g in Try at 10, 30, and 60 min plotted on a semilogarithmic scale.
§ At 10 min after injection, unmetabolized Try-^{14}C represented 12·7% of the injected dose in the brain of one normal rat (209 × 10^3 cpm/g) and 18·8% in the brains of hepatectomized rats. Dose was 15·6 μg/brain (1·7 g) or 9·2 μg/g.

between 10 and 60 min was similar in normal rats and in hepatectomized rats, but the disappearance rate was more than twice as high in the normal rats (Table 6). If complete mixing of injected with endogenous Try is assumed, the turnover of Try in brain (percentage loss times pool size) was 0·44 μg/g/min in normal rats and 0·54 μg/g/min in hepatectomized rats.

Radioactivity in the protein fraction remained almost constant between 30 and 60 min after injection and was slightly but not significantly less than in normal brains. When corrections were made for differences in pool size of Try in brain (complete penetration and mixing of the injected dose with endogenous Try was assumed), a ratio of 1·20 was found as an approximation for the incorporation of Try-^{14}C into brain proteins of hepatectomized rats compared to normal rats in 30 min (Table 7).

The amount of radioactivity in 5-HT in brain of hepatectomized rats was similar to that in normal rats (Table 6). After corrections for differences in pool size, a ratio of 1·76 was found for formation of 5-HT (by hydroxylation and decarboxylation of Try) in brain of hepatectomized rats compared to normal rats in the 30 min after injection (Table 7).

Radioactivity in 5-HIAA was slightly higher in brain of hepatectomized rats than in brain of normal rats (Table 6). Together, 5-HT and 5-HIAA accounted for 0·88% of the administered dose at 30 min and 0·98% at 60 min after injection.

TABLE 7. EFFECT OF HEPATECTOMY ON METABOLISM OF TRY-^{14}C IN RAT BRAIN

	Normal rats	Hepatectomized rats
Free Try in brain:		
endogenous (μg/g)*	6·6	19·9
dose Try-^{14}C (μg/g)†	9·2	9·2
total (μg/g)	15·8	29·1
relative pool size	1	1·84
relative sp. act.	1	0·54
30-Min incorporation of Try-^{14}C into rat brain proteins:		
cpm/g × 10^3	83·6	54·1
relative radioactivity	1	0·65
ratio, relative radioactivity in protein/relative sp. act. of free Try		0·65/0·54 = 1·20
30-Min formation of 5-HT from Try-^{14}C in brain:		
cpm/g × 10^3	8·0	7·6
relative radioactivity	1	0·95
ratio, relative radioactivity in 5-HT/relative sp. act. of free Try		0·95/0·54 = 1·76

* Data from Table 2.

† Try-^{14}C, 15·6 μg, was injected into 1·7 g of brain; complete penetration assumed, this would be equivalent to 9·2 μg/g.

COMMENT

Try concentrations after hepatectomy. When glucose is the only source of nourishment provided to the experimental animals, the source of Try and Tyr in plasma, brain, and other tissues after hepatectomy must be proteins in the extrahepatic tissues. Since Miller[23] has shown that both of these amino acids are metabolized chiefly by the liver, their accumulation after hepatectomy provides some measure of the magnitude of protein breakdown. Protein synthesis also continues after hepatectomy;

the incorporation of Try into brain proteins after direct injection of Try-^{14}C in the brain appeared to be somewhat greater in hepatectomized than in normal rats (Tables 6 and 7).

The concentrations of Try and Tyr in normal dogs and normal rats reported here (Tables 1 and 2) are similar to those reported by others.[24–28] No regional differences were found in the distribution of Try or Tyr in brain of normal dogs, although Price and West[29] had observed differences in distribution of Try measured by paper chromatography.

In the rat, transport of Try into the brain after intravenous injection was altered after hepatectomy (Fig. 3). The net uptake of Try in brain in 30 min was similar in hepatectomized rats and normal rats, although the entry of Try was slower in the hepatectomized rats. This inhibition of the uptake of Try could be caused by the increased concentrations of Tyr and other amino acids[10] in the plasma of the hepatectomized animal. The uptake of Tyr by the brain had been shown to be inhibited by increased concentrations of other amino acids in plasma.[26]

The rate of disappearance of Try-^{14}C from brain after intradiencephalic injection was approximately twice as great in the normal rat as in the hepatectomized rat (Table 6). Since the concentration of Try in the brain of the hepatectomized rat was almost three times normal, the turnover rate was actually somewhat above normal. The disappearance of free Try-^{14}C was due in large measure to its incorporation into brain proteins as well as to hydroxylation with formation of 5-HT and its metabolite, 5-HIAA, and probably also to efflux of Try from the brain.

5-Hydroxyindole concentrations after hepatectomy. Concurrent increases in the size of the pools of 5-hydroxyindoles and of Try were found only in brain of the hepatectomized rat; the concentrations of 5-HT were actually decreased in blood and heart (Table 4) although the concentrations of Try were increased (Table 2). In the hepatectomized dogs, increased concentrations of 5-HT were found previously[21] in the gastrointestinal tract but only in the pyloric region. After hepatectomy, the concentration of 5-HT and of 5-HIAA increased in brain of the rat (Table 4) and that of 5-HIAA increased significantly in brain of the dog (Table 3). 5-HT has a rapid turnover in brain[22] and some may have been metabolized during the 10 to 15 min required for removal and dissection of the brain of the dog.

In both dog and rat, the increases in concentration of 5-HIAA in brain after hepatectomy were much greater than were those of 5-HT (Tables 3 and 4, Fig.2). Thus, the increased concentration of 5-HT in the brain after hepatectomy probably does not result from reduced monoamine oxidase activity. In a number of the hepatectomized rats, 5-HIAA-^{14}C accumulated after the intradiencephalic injection of Try-^{14}C, suggesting either an increased formation or a reduced efflux of 5-HIAA.

The urinary output of 5-HIAA by the rat decreased after hepatectomy by evisceration (Table 4), undoubtedly owing to the removal of the intestinal tract with the liver. It has been shown previously that urinary 5-HIAA is chiefly of intestinal origin in the dog[30, 31] and in the rat.[32] The 24-hr output of 5-HIAA in urine of hepatectomized dogs was within the normal range (Fig. 1), demonstrating that metabolism of 5-HT to 5-HIAA occurs largely in extrahepatic tissues. Donaldson and co-workers[33] found that patients with damaged livers and with impending or frank hepatic coma also excreted normal amounts of 5-HIAA in urine. They concluded that conjugation of 5-HT occurs chiefly in the liver, and metabolism by monoamine oxidase is in

extrahepatic tissues. In the isolated perfused rat liver, less than 5% of administered 5-HT-^{14}C was recovered as 5-HIAA, and 40%, as conjugated 5-hydroxyindoles.[34]

Metabolism of Try in normal and hepatectomized rats. In normal rats the concentration of 5-HT in brain increased after the intravenous injection of much smaller amounts of Try than those used by Weber and Horita[6] (Table 5). However, these investigators gave Try by intraperitoneal injection and measured the concentration of 5-HT in the brain at 2 hr after injection. Because the concentration of the precursor Try in brain decreases at 40 min after injection (Fig. 3), we measured the 5-HT concentration at earlier intervals.

It was possible to increase the concentration of 5-HT and 5-HIAA significantly in brain of normal rats by i.v. administration of Try (33 mg/kg) but this produced only marginal increases in brain of hepatectomized rats (Table 5). It is probable that the concentration of this amine in the brain is maintained within certain limits, either by feedback mechanisms or by saturation of binding sites,[35, 36] and that also these limits are approached in the hepatectomized animal. Weber and Horita[6] injected Try at doses of 200 mg/kg in rats 2 hr after evisceration and obtained a significant increase in concentration of 5-HT in brain. In their eviscerated rats without Try injection, the concentration of 5-HT was not increased above normal.

After the intradiencephalic injection of L-Try-3-^{14}C, radioactive 5-HT was found in brains of both normal and eviscerated rats, indicating that the brain is independent of liver and gastrointestinal mucosa in the hydroxylation of Try. Hydroxylation of Try *in vivo* was first shown to occur in the brain of normal rats by Gal and associates.[4, 5] In our study, after intradiencephalic injection of Try-3-^{14}C, hydroxylation and decarboxylation of Try in brain appeared to occur more rapidly after hepatectomy (Tables 6 and 7). Because of the rapid turnover of 5-HT in brain, measurements of radioactivity in 5-HT at earlier time intervals would be desirable for a more precise comparison.

5-Hydroxyindoles and hepatic coma. Hepatic coma in dogs cannot be attributed to a decreased concentration of 5-HT in the brain, as previously suggested by Borges and co-workers,[1] because a decrease does not occur (Fig. 2). Moreover, the symptoms of hepatic coma in dogs were not altered when the concentration of 5-HT was increased by infusion of 5-HTP or decreased by infusions of dopa. Because dopa and 5-HTP are decarboxylated by the same enzyme,[37] the decrease in 5-HT concentration after administration of dopa (Fig. 2) probably resulted from substrate competition.

Dawson and Sherlock[38] thought that accumulation of amines might be important in the production of hepatic coma. They suggested that the increased concentration of ammonia in blood of patients with liver disease might be due to the action of monoamine oxidase on amines. Because the increases in 5-hydroxyindole concentration in our hepatectomized dogs were found only in brain and in a small region of the gastrointestinal tract, it is unlikely that they could make a significant contribution to the blood ammonia level. No increases were found in the concentration of another amine, tryptamine, in plasma or brain of rats or dogs after hepatectomy.

Increased concentrations of 5-HT in the brain are not usually associated with coma but with hyperactivity and agitation. Although the increased concentrations of 5-HT in brain may be a contributing factor, hepatic coma is obviously a complex entity in which many other factors are of greater importance. Ammonia metabolism is disordered,[39] and the metabolism of glucose in brain is altered, in hepatic coma.[40]

Alterations in transport of substances into the brain may also be important in the development of coma.

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