Biochemical Pharmacology, Vol 17, pp 1543–1552. Pergamon Press. 1968. Printed in Great Britain

METABOLISM OF SEROTONIN BY THE ISOLATED PERFUSED RAT LIVER—EFFECT OF GLUCURONYL TRANSFERASE DEFICIENCY OR MONOAMINE OXIDASE INHIBITION*

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(*Received* 4 *December* 1967; *accepted* 15 *March* 1968)

Abstract—The metabolism of 5-hydroxytryptamine-3′-^{14}C (5-HT, serotonin) was studied in isolated perfused rat livers when the monoamine oxidase pathway was inhibited by β-phenylisopropylhydrazine (JB 516) or the glucuronyl transferase pathway was deficient (Gunn rat livers). In all cases, 5-HT was cleared rapidly from the plasma by liver and platelets. In bile and in plasma in control perfusions (Sprague–Dawley livers), monoamine oxidase metabolites [chiefly conjugates of 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptophol (5-HTOH)] accounted for 29 per cent of the dose; conjugates of *N*-acetyl-5-hydroxytryptamine accounted for 12 per cent of the dose and 5-hydroxytryptamine glucuronide for 11 per cent. On addition of 2 mg JB 516 to the perfusate, the amounts of conjugates of 5-HT and *N*-acetyl-5-hydroxytryptamine increased and no radioactive 5-HTOH or 5-HIAA could be detected. Gunn rat livers produced less glucuronides, more sulfate conjugates and more free 5-HIAA compared to perfusion of Sprague–Dawley livers without JB516. The total amount of 5-HT metabolized was not reduced when a major metabolic pathway was either deficient or inhibited, because compensatory increases occurred in the amount metabolized by alternate pathways.

THE ABILITY of the liver to rapidly metabolize serotonin (5-hydroxytryptamine, 5-HT) has been demonstrated previously[1] in the isolated perfused rat liver system. When 27 μg 5-HT-^{14}C was injected into blood entering the portal vein, the liver took up more than 50 per cent of the injected dose. The remaining 5-HT-^{14}C was taken up very rapidly by platelets in the perfusate and was protected from metabolism by the liver. As a result of the uptake by platelets and by liver, 5-HT-^{14}C was not detectable in plasma 5 min after injection.

The liver metabolized the amine by multiple pathways and released metabolites into the bile and the plasma. About 26 per cent of the injected 5-HT-^{14}C was metabolized via the monoamine oxidase (MAO) pathway to form chiefly glucuronide or sulfate conjugates of 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptophol (5-HTOH) and the sulfate of a compound thought to be 5-hydroxyindoleacetaldehyde (5-HIAc). Only 6 per cent of the injected radioactivity was recovered as free 5-HIAA in plasma; 10 per cent of the injected 5-HT-^{14}C was conjugated with glucuronic acid and 12 per cent was subjected to *N*-acetylation and conjugation.

* This investigation was supported in part by Research Grant NB-4004 from the National Institutes of Health, Public Health Service.

In the present experiments, hepatic metabolism of 5-HT was studied under conditions in which one of the major pathways was inhibited or deficient. Inhibition of the MAO pathway was brought about by the addition to the perfusate of the MAO inhibitor, β-phenylisopropylhydrazine (JB 516). Metabolism of 5-HT was also studied in livers from Gunn rats, a strain deficient in glucuronyl transferase and jaundiced because of their inability to conjugate bilirubin.[2,3] A preliminary report of these investigations has been made.[4]

METHODS

Materials

The following compounds were purchased: 5-HIAA, 5-methoxytryptamine, *N*-acetyl-5-methoxytryptamine (melatonin) and *N,N*-dimethyl-5-hydroxytryptamine (bufotenine) from the Regis Chemical Co. (Chicago); 5-HTOH and *N,N*-dimethyl-5-methoxytryptamine from Orion Laboratories, Inc. (Cleveland); *N*-acetyl-5-hydroxytryptamine (N-acetylserotonin; NAS) from Mann Research Laboratories (New York); and *N*-methyl-5-hydroxytryptamine from the Aldrich Chemical Company (Milwaukee). 5-Methoxyindoleacetic acid was generously supplied by Sandoz Pharmaceuticals (Hanover, N.J.). Dehydrobufotenine, isolated as the natural product, was the gift of Dr. W. F. Gannon of Regis Chemical Company. 5-HT-3′-^{14}C creatinine sulfate (sp. act., 39·6 mc/m-mole) was obtained from Nuclear-Chicago (Des Plaines, Ill.).

The method used for the perfusion of the liver was that described by Brauer *et al.*[5] as modified by Flock and Owen.[6] A rat liver was perfused with a mixture of 100 ml rat blood and 20 ml isotonic saline for 60 min to remove extraneous pressor substances from the perfusate. This liver was then discarded and a second liver was connected to the system. The second liver (the actual experimental liver) was perfused for 5 hr.

In all experiments, the injection of 5-HT-3′-^{14}C (5 μc, 27 μg) was made directly into the blood entering the portal vein of the experimental liver. In all experiments, the injection of 5-HT caused a brief reduction in blood flow through the liver. The methods used for sampling and measurements of radioactivity in bile, whole blood and plasma have been described previously.[1] Liver ^{14}C content was calculated as the difference between the injected dose and the sum of the ^{14}C in whole blood and bile. Metabolites in bile, in plasma and in liver were separated by paper chromatography, TLC or high-voltage paper electrophoresis.[1] Methods for identification and determination of the distribution of radioactivity in metabolites have been described previously.[1]

In a number of experiments, livers from Sprague–Dawley rats weighing 300–370 g were perfused. The mean (± S.E.) weight of the livers was 11·0 ± 0·3 g.

In some experiments, either 2 or 0·2 mg of the drug JB 516 (dissolved in 3 ml isotonic saline) was added to the perfusate after the removal of the first liver. The experimental liver was then connected to the system, and the 5-HT-^{14}C was injected 30 min after the addition of the JB 516. The mean weight of the livers (Sprague–Dawley rats) was 12·0 ± 0·3 g.

In other experiments, livers from rats of the Gunn strain, weighing 250–425 g, were perfused. The mean weight of the livers was 10·1 ± 0·4 g. Blood from normal (Sprague–Dawley) rats was used for the perfusate in these experiments also.

RESULTS

Disposition of radioactivity after injection of 5-HT-3′-^{14}C

Much of the injected 5-HT-3′-^{14}C was taken up by the liver (Fig. 1). At 5 min after injection, 52 per cent of the dose was found in the liver in control experiments. The decrease of ^{14}C in normal livers was biphasic: a rapid release occurred in the first 30 min, followed by a slower release. The decrease after 30 min appeared to be exponential; the fractional rate of decrease was 0·55 per cent per min and the half-time of ^{14}C in the liver was 126 min. After 5 hr, less than 10 per cent of the dose remained in the liver.

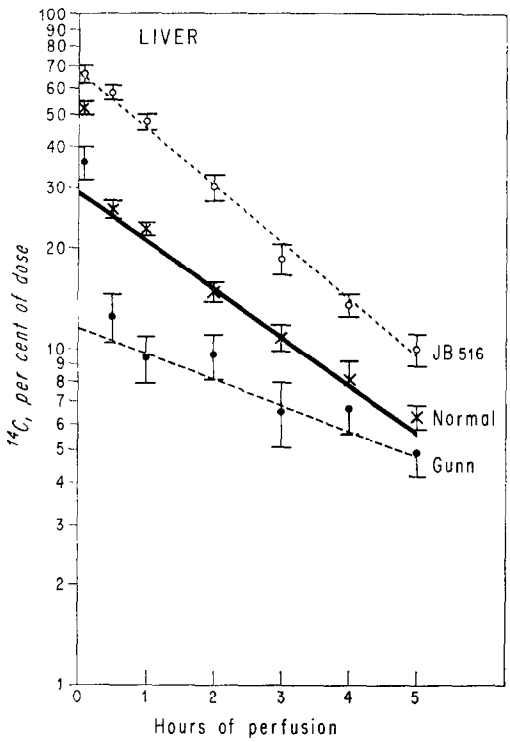

FIG. 1. Total ^{14}C in liver (calculated by subtracting total ^{14}C in bile and in plasma from the total injected ^{14}C) at different times after injection of 5 μc 5-HT-3′-^{14}C in isolated rat liver perfusions (16 perfusions of normal livers, 5 perfusions after addition of 2 mg JB 516, and 6 perfusions of livers from Gunn rats). Vertical lines show one standard error of the mean. Lines of best fit (calculated by the method of least squares) are shown for values after 30 min in perfusions of livers from normal and from Gunn rats and for values at all time intervals in perfusion with added JB 516.

In perfusions with 2 mg JB 516 added, a monophasic exponential decrease of ^{14}C in liver occurred at a fractional rate of 0·66 per cent per min; the half-time was 105 min. By extrapolation it could be estimated that, at the time of injection, 68 per cent of the dose was in the liver; after 5 hr of perfusion about 10 per cent of the dose remained in the liver.

Although significantly more ^{14}C was present in the liver at all time intervals when 2 mg JB 516 had been added, the slope of disappearance of ^{14}C from the liver in JB 516 perfusions was similar to that of the second component of the ^{14}C decrease in

control perfusions. Thus, from 30 min to 5 hr after injection, ^{14}C in liver decreased at almost the same rate in the presence as in the absence of JB 516.

In perfusions of Gunn rat livers, less ^{14}C was present in the liver at all time intervals compared to the normal livers ($P < 0.05$). As in normal livers, the decrease in ^{14}C was biphasic. A rapid decrease occurred in the first 30 min. After 30 min the decrease was slower and somewhat erratic, but appeared to be at the rate of 0·28 per cent per min; the half-time was 240 min. After 5 hr, less than 5 per cent of the dose remained in the liver.

In normal perfusions, ^{14}C in whole blood and in plasma increased during the perfusion, most of the increase occurring in the first hour (Fig. 2, A and B). After 5 hr of perfusion, 70 per cent of the radioactivity was in whole blood and 38 per cent was in plasma. With 2 mg JB 516 added to the perfusate, the amounts of ^{14}C in whole blood and in plasma were significantly less than in controls ($P < 0.01$) during the first 2 hr of perfusion. After 2 hr, the differences from control values were not significant. More ^{14}C ($P < 0.001$) was found in whole blood and in plasma in perfusions of Gunn rat livers than in normal perfusions.

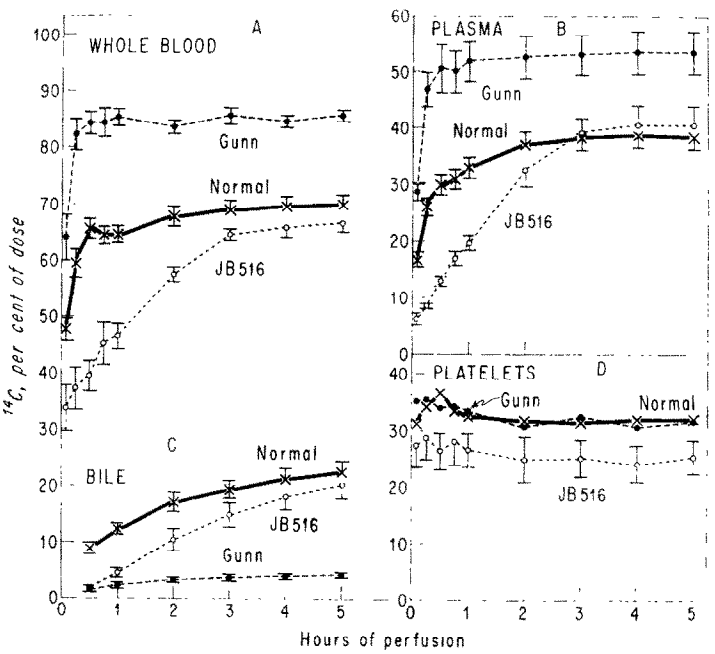

FIG. 2. Total ^{14}C in whole blood (A), plasma (B), bile (C) and platelets (D) at different times after endoportal injection of 5 μc 5-HT-3′-^{14}C in isolated rat liver perfusions. Means (± S.E.) of 16 normal perfusions, 5 perfusions after the addition of 2 mg JB 516, and 6 perfusions of livers from Gunn rats are shown. Where S.E. is not shown, it was too small to be represented graphically.

Measurements of ^{14}C in platelet-rich plasma and in platelet-poor plasma indicated that the differences between ^{14}C in whole blood and ^{14}C in plasma represented platelet-bound ^{14}C. The amount of platelet-bound ^{14}C remained almost constant throughout normal perfusions, JB 516 perfusions and Gunn rat liver perfusions (Fig. 2, D). Somewhat less ^{14}C was bound to platelets in the JB 516 perfusions than in controls,

but the differences were not significant except at 30 min. In Gunn rat liver perfusions the amount of platelet-bound ^{14}C was not different from normal.

In normal perfusions 22·5 per cent of the dose was excreted in bile (Fig. 2, C). When 2 mg JB 516 was added, biliary ^{14}C was significantly less than in controls during the first 2 hr of perfusion; at later time intervals, the differences were not significant. Gunn rat livers excreted much less ^{14}C in bile than did the normal livers.

Metabolites of 5-HT-^{14}C in bile and in plasma

Normal Perfusions. No free ^{14}C-labeled 5-HT or 5-HIAA was detected in bile; the radioactivity was found chiefly in glucuronides, the most abundant compound being the glucuronide of NAS (6·3 per cent of the dose; Table 1, Fig. 3). ^{14}C was also present in glucuronides of 5-HTOH, 5-HIAA and 5-HT. Radioactivity in NAS sulfate accounted for 0·9 per cent of the dose; trace radioactivity ($< 0·3$ per cent of the dose) in 5-HTOH sulfate was detected in a few perfusions. Traces of other radioactive substances were found occasionally in bile, but the total radioactivity in these compounds did not exceed 2 per cent of the dose and their identification was not attempted. The sum of the radioactivity in identified compounds accounted for 75 per cent of the total radioactivity in bile; the remaining radioactivity represented losses during separation, particularly radioactivity left at the origin in the TLC separation of NAS and 5-HTOH after the enzymic hydrolysis of their conjugates. Co-chromatography with reference standards failed to detect any of the following compounds, free or conjugated: 5-methoxytryptamine, *N*-acetyl-5-methoxytryptamine, *N*-methyl-5-hydroxytryptamine, bufotenine, 5-methoxytryptophol and *N,N*-dimethyl-5-methoxytryptamine.

In plasma no radioactivity was detected as free 5-HT after 5 min, 1 hr or 5 hr of perfusion (Fig. 4). Radioactivity as free 5-HIAA accounted for only 6·7 per cent of the dose (Table 1). The remaining radioactivity was found as glucuronides or sulfates of 5-HIAA, 5-HTOH, 5-HT and NAS. Also present in plasma, accounting for 4 per cent of the dose, was the sulfate of a compound thought to be 5-HIAc.

Perfusions with JB 516 added. When 2 mg JB 516 was added to the perfusate, radioactivity was not detectable as 5-HIAA or its conjugates, as 5-HTOH conjugates or as the sulfate of 5-HIAc (Table 1). Radioactivity in 5-HT glucuronide and in NAS glucuronide in bile and in plasma was increased. No increases were observed in the amounts of unidentified radioactive compounds. As in control perfusions, no radioactivity was associated with 5-HT after 5 min, 1 hr or 5 hr of perfusion.

When 0·2 mg JB 516 was added to the perfusate (two perfusions, not illustrated), differences from control perfusions were much smaller, but were similar to those produced by the higher dose. ^{14}C in free 5-HIAA was reduced to 3 per cent and 4 per cent of the injected dose. However, total metabolites resulting from MAO activity accounted for 21 per cent and 28 per cent of the dose, compared to the 29·4 per cent in control perfusions.

Perfusions with livers from Gunn rats. When Gunn rat livers were perfused, the amounts of all radioactive glucuronides, except 5-HT glucuronide, in bile were reduced (Table 1, Fig. 3). The amount of radioactive NAS sulfate was also reduced. There were no increases in any of the unidentified radioactive compounds.

In plasma, no radioactive 5-HT was present after 5 min, 1 hr or 5 hr of perfusion. The amount of ^{14}C in 5-HT glucuronide was reduced and the amount in free 5-HIAA

TABLE 1. DISTRIBUTION OF ^{14}C AFTER INJECTION OF 5-HT-3′-^{14}C IN ISOLATED PERFUSED RAT LIVER SYSTEM

Compound	^{14}C (% of dose)*					
	Bile			Perfusate plasma		
	Normal (n = 13)†	JB 516 (n = 5)	Gunn rats (n = 6)	Normal (n = 13)†	JB 516 (n = 5)	Gunn rats (n = 6)
Total ^{14}C	19·5 ± 1·6	20·3 ± 2·3	4·3 ± 0·6 (< 0·001)‡	38·3 ± 2·1	41·3 ± 3·4	53·7 ± 3·8 (< 0·001)
5-HTOH glucuronide	3·4 ± 0·4	0	0·1 ± 0·02 (< 0·05)	2·6 ± 0·6	0	2·1 ± 0·9
5-HTOH sulfate	0·1 ± 0·03	0	0·2 ± 0·03	2·0 ± 0·1	0	3·2 ± 0·2 (< 0·001)
5-HIAc sulfate	0	0	0	4·0 ± 0·5	0	5·9 ±1·0
5-HIAA, free	0	0	0	6·7 ± 0·6	0	20·4 ± 2·5 (< 0·001)
5-HIAA glucuronide	1·9 ± 0·3	0	0·4 ± 0·2 (< 0·01)	0·8 ± 0·2	0	0·4 + 0·2
5-HIAA sulfate	0	0	0	7·9 ± 1·1	0	11·5 ± 1·1 (< 0·05)
MAO metabolites (sum)	5·4	0	0·7	24·0	0	43·5
5-HT glucuronide	2·2 ± 0·5	5·5 ± 1·2 (< 0·01)	1·6 ± 0·4	9·1 ± 0·9	29·7 ± 1·9 (< 0·001)	4·1 ± 1·1 (< 0·01)
NAS glucuronide	6·3 ± 0·9	10·5 ± 1·3 (0·05)	0·5 ± 0·1‡ (< 0·05)	4·3 ± 0·9	8·3 ± 1·3 (< 0·05)	2·1 ± 0·4
NAS sulfate	0·9 ± 0·1	1·3 ± 0·4	0·6 ± 0·1‡ (< 0·05)	0·3 ± 0·1	0·3 ± 0·2	0·9 ± 0·3 (< 0·05)
Glucuronide conjugates (sum)	13·8	16·0	2·6	16·8	38·0	8·7
Sulfate conjugates (sum)	1·0	1·3	0·8	14·2	0·3	21·5

* Shown as means ± S.E.

† Perfusions of normal livers include some data previously published.[1]

‡ In parentheses are shown P values for differences from controls.

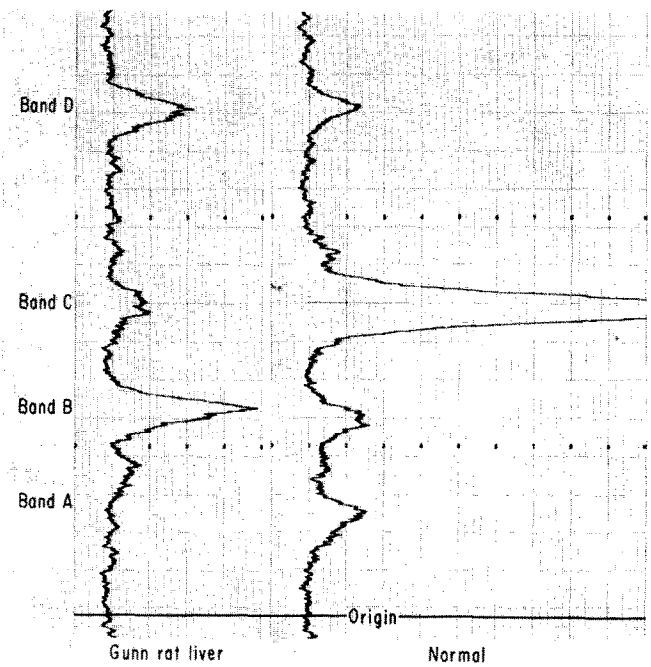

FIG. 3. Radioactivity on a one-dimensional chromatogram (solvent, *n*-butanol–pyridine–water; 1:1:1) of bile from normal and Gunn rat liver perfusions. Bands are: A, glucuronide of 5-HIAA; B, glucuronide of 5-HT; C, mixture of glucuronides of NAS and 5-HTOH; and D, mixture of sulfates of NAS and 5-HTOH.

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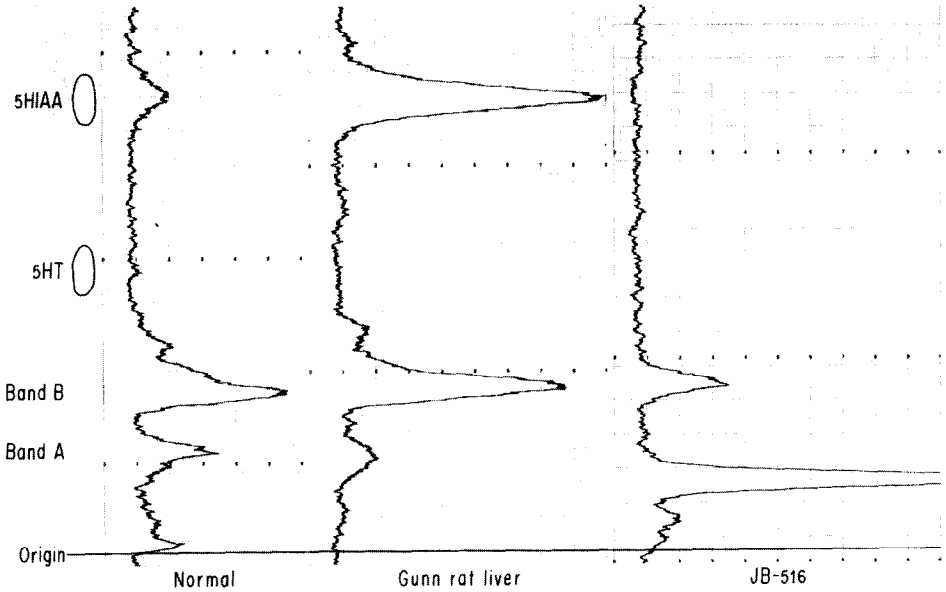

FIG. 4. Radioactivity on one-dimensional chromatograms (solvent, *n*-butanol–acetic acid–water; 120:30:50) of methanol–acetone extracts of plasma after 5 hr of perfusion of isolated livers. Positions of markers are shown at the side of the radioactivity tracings. In butanol–pyridine–water, band A separates into 5-HT glucuronide and 5-HIAc sulfate and band B separates into 5-HIAA glucuronide, 5-HIAA sulfate, NAS glucuronide, NAS sulfate, 5-HTOH glucuronide and 5-HTOH sulfate.

was increased (Table 1, Fig. 4). Radioactivity associated with the sulfates of 5-HIAA, 5-HTOH and NAS also was increased. Other differences from normal perfusions were not significant.

Metabolites of 5-HT-3′-^{14}C in liver

Five hr after the injection of 5-HT-^{14}C, 6·9 per cent of the dose remained in the liver in normal perfusions; 4·0 per cent was extractable by methanol–acetone. On paper chromatography in *n*-butanol–acetic acid–water (120:30:50), the distribution of radioactivity in methanol–acetone extracts of liver resembled that in plasma extracts.[1] Much of the ^{14}C was found as unmetabolized 5-HT (1·5 per cent of dose; Table 2); also present was radioactive 5-HIAA and two radioactive bands, A and B, between 5-HT and the origin. Because the percentage of radioactivity in bands A and B was small, resolution of components and individual measurement of associated radioactivity were not usually attempted.

In one control experiment terminated after 1 hr of perfusion, 22·6 per cent of the dose was in the liver; more than half of this precipitated with the proteins. Most of the radioactivity (5·2 per cent of the dose) in the methanol–acetone extract was associated with band A, which was found to contain only 5-HT glucuronide.

With 2 mg JB 516 added to the perfusate, there was more ^{14}C present in the liver and radioactive 5-HIAA was absent, but otherwise the distribution of radioactivity on paper chromatograms of methanol–acetone extracts of liver was essentially the same as in the controls. In one experiment with 2 mg JB 516, terminated after 1 hr of perfusion, 48·9 per cent of the injected ^{14}C was in the liver (almost half of this was protein bound); most of the ^{14}C extracted into methanol–acetone was associated with 5-HT glucuronide (19·0 per cent of the dose). Also present were smaller amounts of radioactive NAS glucuronide, NAS sulfate and unmetabolized 5-HT.

With Gunn rat livers, after 5 hr of perfusion, somewhat less ^{14}C was present in the liver compared to normal livers, but the distribution of radioactivity was similar.

DISCUSSION

The metabolism of 5-HT-^{14}C by the liver (together with the release of ^{14}C-labeled metabolites) has been shown to have two phases: an initial rapid component and a slower component. The initial rapid component was dominant in perfusions using livers from Gunn rats and was absent in perfusion of normal livers with MAO inhibitor added. Because MAO metabolites (particularly free 5-HIAA) were formed abundantly by the Gunn rat livers and not at all in normal livers after addition of inhibitor, it would seem that the initial rapid phase represents MAO activity and the slower phase represents *N*-acetylation and conjugation. Metabolism by MAO appears to be more rapid than *N*-acetylation or conjugation. However, the presence of large amounts of radioactive 5-HT glucuronide in liver after 1 hr of perfusion suggests that conjugation may be just as rapid as monoamine oxidation, but that conjugated metabolites are released from the liver more slowly than are MAO metabolites.

For effective inhibition of MAO, a high dose (2 mg) of JB 516 was required. The addition of 0·2 mg JB 516 (equivalent to about 1·5 mg/kg) had little effect, whereas in experiments *in vivo* Horita[7] found that MAO activity in homogenates of livers from rats treated with $7{\cdot}5 \times 10^{-6}$ mole/kg (about 1·4 mg/kg) was reduced by 70–80 per cent. However, hepatic metabolism of the drug undoubtedly occurs. In other studies,

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN LIVER AFTER PORTAL INJECTION OF 5-HT-3′-^{14}C IN ISOLATED PERFUSED RAT LIVER

Experimental conditions	Perfusion time (hr)	^{14}C (% of dose)*						
		Band A†	Band B‡	5-HT	5-HIAA	Free (methanol–acetone extract)	Bound (total – free)	Total (entire homogenate)
Normal§ (n = 9)	5	1·0 ± 0·1	1·2 ± 0·1	1·5 ± 0·2	0·3 ± 0·02	4·0 ± 0·2	2·9 ± 0·2	6·9 ± 0·4
Normal (n = 1)	1	5·2‖	2·6	1·8	0·5	10·1	12·5	22·6
Added JB 516 (n = 5)	5	2·3 ± 0·3	0·9 ± 0·3	2·2 ± 0·3	0	5·4 ± 0·5	4·6 ± 0·7	10·1 ± 1·2
Added JB 516 (n = 1)	1	19·0‖	3·9¶	4·8	0	28·0	20·9	48·9
Gunn rat liver (n = 6)	5	0·6 ± 0·1	1·0 ± 0·1	1·3 ± 0·2	0·8 ± 0·1	3·4 ± 0·2	1·2 ± 0·2	4·6 ± 0·3

* Data are shown as means ± S.E.

† On paper chromatography in *n*-butanol–pyridine–water (1:1:1), band A separates into 5-HT glucuronide and 5-HIAc sulfate.

‡ On paper chromatography (above system), band B separates into 5-HIAA glucuronide, 5-HIAA sulfate, NAS glucuronide, NAS sulfate, 5-HTOH glucuronide and 5-HTOH sulfate.

§ Control experiments include some data previously published.[1]

‖ Only 5-HT glucuronide present.

¶ Only NAS glucuronide and NAS sulfate present.

for complete inhibition of monoamine oxidation of norepinephrine-^{14}C in the isolated perfused rat liver, it was necessary to infuse much larger doses of the inhibitor (2·5 mg/hr) throughout the duration of perfusion.[8]

The addition of the MAO inhibitor decreased somewhat the amount of 5-HT-^{14}C taken up by platelets, but increased uptake by the normal liver. 5-HT-^{14}C was cleared from the plasma just as rapidly as in the control perfusions. Although the early excretion of metabolites was slower when MAO inhibitor had been added, the total amount of 5-HT-^{14}C metabolized was essentially the same in the presence or absence of the drug. Addition of 2 mg JB 516 completely inhibited the MAO pathway; the amounts of 5-HT glucuronide and of NAS conjugates in bile and in plasma were significantly increased. Thus, although MAO inhibitors of the hydrazine type can inhibit enzymes other than MAO,[9] in the present experiments no inhibition by JB 516 of pathways of 5-HT metabolism other than monoamine oxidation could be demonstrated.

In perfusions of livers from Gunn rats, 5-HT was also cleared rapidly from plasma and was not detectable after 5 min. Liver uptake of 5-HT-^{14}C at 5 min after injection was less in Gunn rat livers than in normal livers. However, it is likely that the initial uptake was not less in the Gunn rat livers because metabolism by Gunn rat livers occurred chiefly in the first 30 min and probably considerable metabolism and release had occurred during the first 5 min.

The livers of Gunn rats did not form glucuronides of 5-hydroxyindoles in appreciable amounts. Compensatory increases were found in the amount of free 5-HIAA-^{14}C in plasma and, to a lesser extent, in the amounts of labeled sulfate conjugates. In the present experiments the amount of NAS glucuronide formed by livers of Gunn rats was decreased compared to that formed by normal livers, without compensatory increases in free NAS or in NAS sulfate; thus, livers of Gunn rats may differ from those of Sprague–Dawley rats in other respects in addition to the well known deficiency of glucuronyl transferase. Either the Gunn rat liver cannot *N*-acetylate 5-HT or the formation of 5-HT glucuronide normally precedes *N*-acetylation.

5-HT-^{14}C was very rapidly metabolized by the isolated perfused rat liver by three major pathways: monoamine oxidation, *N*-acetylation and conjugation. When one of these pathways was repressed, compensatory changes were found in other pathways. Although there were a number of minor radioactive compounds not identified in the control experiments,[1] the amounts of these were not increased when either enzyme was deficient or inhibited. Also, it was not possible to detect any unusual metabolite of 5-HT, such as the hallucinogenic compounds bufotenine or *N*-methyl-5-hydroxytryptamine, even when one of the major metabolic pathways was repressed. Further experiments are needed to determine whether such compounds might be detected when two metabolic pathways are repressed, as would be the case in Gunn rat livers perfused with JB 516.

Acknowledgements—Mr. Emery Van Hook, Mrs. Avis Gray and Mr. Donald Gustafson provided valuable technical assistance.

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