*Br. J. Pharmac.* (1971), **42**, 273–280.

# 5-Hydroxytryptamine metabolism in sheep

A. L. BARTLET

*Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, Edinburgh*

**Summary**

1. 5-Hydroxytryptamine (5-HT) (10 mg) administered intravenously to sheep was mostly excreted in the urine as 5-HT-*O*-glucuronide (705 ± 162 μg, mean from six wethers ± S.E.M.) and 5-hydroxyindolylacetic acid (5-HIAA) (6,850 ± 1,538 μg). Oral administration of 5-HT (100 mg) led to the excretion of 590 ± 212 μg 5-HT-*O*-glucuronide (mean from five wethers ± S.E.M.) and 7,394 ± 2,093 μg 5-HIAA in the urine.

2. After administration of DL-5-hydroxytryptophan (DL-5-HTP) (20 mg i.v. or 200 mg orally) sheep excreted about 5 mg 5-HT in the urine together with 5-HT-*O*-glucuronide and 5-HIAA. The excretion of 5-HT suggests that some DL-5-HTP perfused the liver and the lungs before being decarboxylated.

3. Twenty-four hour specimens of control urine contained 335 ± 45 μg 5-HT, 909 ± 90 μg 5-HT-*O*-glucuronide and 3,352 ± 362 μg 5-HIAA (seven sheep in each instance). Thus endogenous 5-HT seemed to be conjugated with glucuronic acid to a greater extent than administered 5-HT.

4. Although urinary 5-HIAA was reduced after a single dose of isocarboxazid (20 mg/kg daily) the 5-HT-*O*-glucuronide content of the specimens was not increased until two or three doses of the drug had been given.

5. Carbon tetrachloride (4 ml orally) reduced the urinary excretion of 5-HT-*O*-glucuronide. Although the conjugation of 5-HT with glucuronic acid was inhibited after administration of carbon tetrachloride the excretion of 5-HT and 5-HIAA was not increased. This observation suggests that carbon tetrachloride impaired the formation or clearance of endogenous 5-HT in sheep.

## Introduction

Endogenous 5-hydroxytryptamine (5-HT) seems to be metabolized by two routes in sheep as substantial amounts of 5-hydroxyindolylacetic acid (5-HIAA) and 5-HT-*O*-glucuronide are excreted in the urine (Bartlet & Gilbert, 1971). The present experiments were carried out in order to investigate more fully the ability of monoamine oxidase and UDP-glucuronyl transferase to metabolize 5-HT in this animal. Estimates have been made of the urinary metabolites from 5-HT and DL-5-hydroxytryptophan (DL-5-HTP) administered to sheep by the oral and intravenous routes, and the effects of isocarboxazid and carbon tetrachloride on the urinary metabolites of endogenous 5-HT have been examined.

## Methods

### *Animals*

Seven black faced wethers, body weight 26·9 ± 2·3 kg (mean ± S.E.M.), were kept on a diet of oats (150 g/day), unrestricted hay and water. The sheep were given

thiabendazole (2–3 g), which was repeated when nematode eggs were found in samples of the faeces. The eggs of liver flukes were never found in the faecal samples. The method used for the collection of specimens of urine has been described (Bartlet & Gilbert, 1971).

### *Drugs*

Capsules of carbon tetrachloride B.Vet.C. (1 ml) (Boots Ltd.), 5-hydroxyindolylacetic acid diethylammonium salt (B.D.H.), 5-hydroxytryptamine creatinine sulphate (B.D.H.), DL-5-hydroxytryptophan, isocarboxazid (marplan) and thiabendazole (Merck, Sharp & Dohme Ltd.) were used. 5-HT and DL-5-HTP were dissolved in 0·9% (w/v) aqueous NaCl and injected intravenously or administered orally. Isocarboxazid was suspended in 2% (w/v) aqueous gum acacia for oral administration.

### *Estimation of 5-hydroxyindoles and creatinine*

The methods for the estimation of 5-HT, 5-HT-*O*-glucuronide and 5-HIAA in sheep urine have been described (Bartlet & Gilbert, 1970, 1971). The amounts of 5-HT-*O*-glucuronide in the text, tables and figures have been given in terms of the 5-HT liberated by β-glucuronidase. 5-HT and 5-HIAA have been expressed as weights of free base and acid, respectively. Creatinine was determined by the sodium picrate method after extraction of the urine with peroxide-free ether (Taussky, 1956).

## Results

### *Metabolites of administered 5-HT*

5-HT (10 mg) injected intravenously often produced a brief recumbency and dyspnoea for up to 20 minutes. Neither 5-HT (10 mg) intravenously nor 5-HT (100 mg) orally affected the volume and creatinine content of the urine specimens.

When 5-HT was administered to sheep measurable amounts of its metabolites were excreted in the urine specimen collected in the first 24 h but not in subsequent collections (Fig. 1). Values for the metabolites derived from the administered 5-HT in 24 h specimens of urine are given in Table 1. The administration of 5-HT to sheep led to a significant rise in the excretion of 5-HT-*O*-glucuronide and 5-HIAA but not 5-HT. Both 5-HT (10 mg) intravenously and 5-HT (100 mg) orally produced about 8 mg of urinary metabolites of the drug, about 90% of the metabolites of 5-HT administered intravenously or orally being 5-HIAA.

TABLE 1. *Urinary metabolites of administered 5-hydroxytryptamine*

| Administration of 5-HT | Increase in the metabolites in urine | | *P* |
|---|---|---|---|
| 10 mg (i.v.) | 5-HT | 355±200 μg | >0·1 |
| | 5-HT-*O*-glucuronide | 705±162 μg | <0·01 |
| | 5-HIAA | 6850±1538 μg | <0·01 |
| 100 mg (oral) | 5-HT | 30±19 μg | >0·1 |
| | 5-HT-*O*-glucuronide | 590±212 μg | <0·05 |
| | 5-HIAA | 7394±2093 μg | <0·05 |

Increase in the excretion of 5-hydroxyindoles in 24 h urine specimens collected after administration of 5-HT. Each value is a mean±S.E.M. obtained from six wethers injected with the drug or five wethers dosed orally.

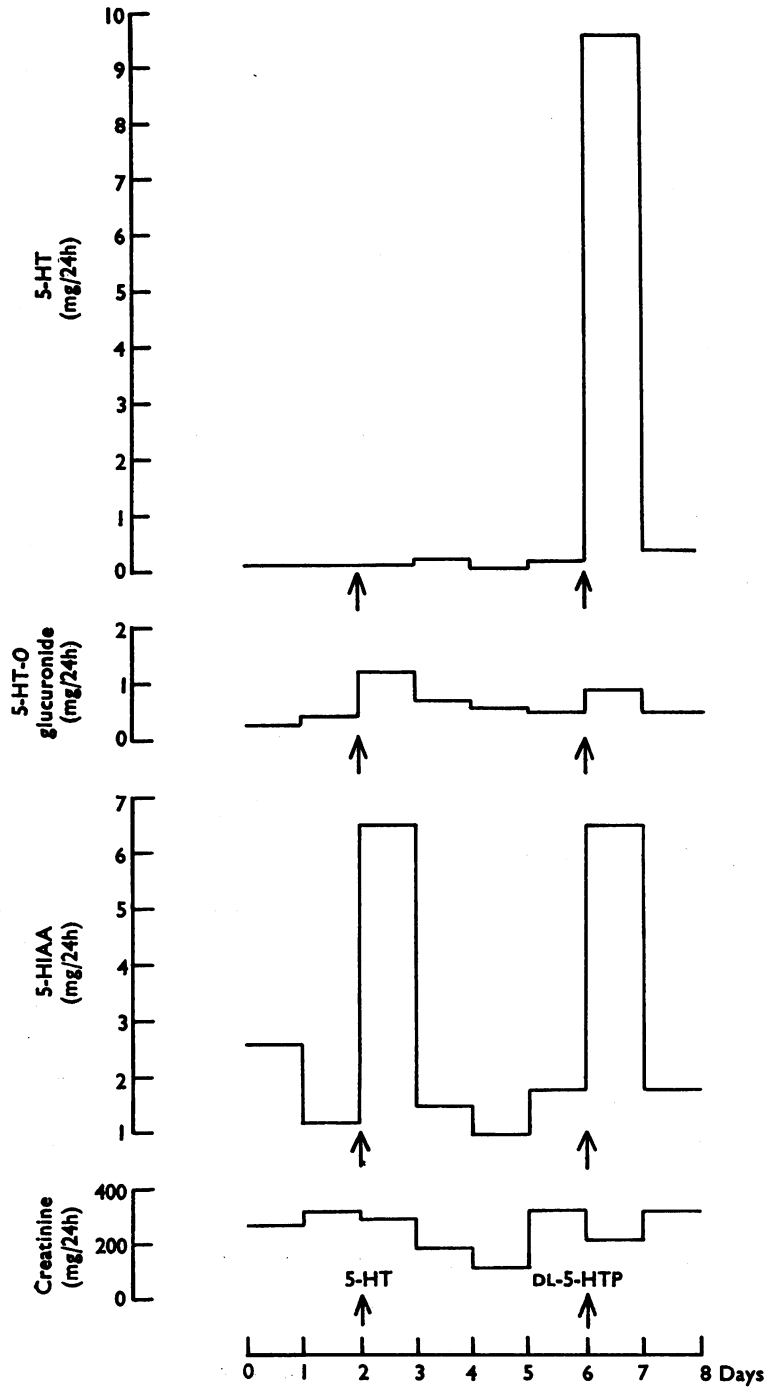


FIG. 1. Excretion of 5-hydroxyindolyl compounds in the urine of the sheep after administration of 5-HT and DL-5-HTP. Sheep, two-two, 19 kg. From above downwards: urinary 5-HT (mg/24 h); 5-HT-*O*-glucuronide (mg/24 h); 5-HIAA (mg/24 h) and creatinine (mg/24 h). The arrows mark the oral administration of 5-HT (100 mg) after 2 days of the experiment and DL-5-HTP (200 mg) after 6 days.

### *Metabolites of administered* DL-*5-HTP*

DL-5-HTP was administered in doses which were twice those used for 5-HT as only the L-isomer of the amino-acid is decarboxylated to 5-HT by 5-HTP decarboxylase. Intravenous or oral administration of DL-5-HTP produced no symptoms in the animals and did not affect the volume or creatinine content of the urine specimens. Measurable amounts of metabolites of administered DL-5-HTP were only excreted in the urine collected in the first 24 h after administration of the amino-acid (Fig. 1). About 5 mg 5-HT were excreted in the urine after administration of DL-5-HTP (20 mg) intravenously or DL-5-HTP (200 mg) orally (Table 2). This observation contrasts with the insignificant effect of similar doses of administered 5-HT on the 5-HT content of the urine. Intravenous or oral administration of DL-5-HTP also produced rises in the urinary excretion of 5-HIAA and 5-HT-*O*-glucuronide which were similar to those found after administration of 5-HT.

### *Metabolites of endogenous 5-HT*

The following values for the 5-hydroxyindoles and creatinine in control urine were calculated from the means for each animal in the foregoing experiments. The 24 h specimens contained $335 \pm 45$ $\mu$g 5-HT, $909 \pm 90$ $\mu$g 5-HT-*O*-glucuronide, $3{,}352 \pm 362$ $\mu$g 5-HIAA and $488 \pm 38$ mg creatinine. The amount of each metabolite of endogenous 5-HT expressed as a percentage of the 5-hydroxyindole content of the specimens was 7% 5-HT, 20% 5-HT-*O*-glucuronide and 73% 5-HIAA. These values are very similar to those obtained previously for sheep urine (Bartlet & Gilbert, 1971). The proportion of endogenous 5-HT conjugated with glucuronic acid was greater than that for administered 5-HT.

### *Effect of isocarboxazid on metabolites of endogenous 5-HT*

Isocarboxazid, an inhibitor of monoamine oxidase, was administered orally to three sheep at a dose of 20 mg/kg on 3 successive days. The sheep became very alert and stamped their hoofs frequently after administration of isocarboxazid. Anorexia was observed after the second dose of the drug, the animals refusing oats and sometimes hay. The sheep passed very few faeces after isocarboxazid and in two experiments the volume and creatinine content of the urine specimens were reduced. The effect of isocarboxazid on the urinary excretion of 5-HT, 5-HT-*O*-glucuronide and 5-HIAA was similar in the three animals used, the results of a typical experiment being shown in Fig. 2.

Isocarboxazid produced a prompt reduction in the urinary excretion of 5-HIAA and after some delay a rise in the 5-HT-*O*-glucuronide content of the specimens.

TABLE 2. *Urinary metabolites of administered* DL-*5-hydroxytryptophan*

| Administration of DL-5-HTP | Increase in the metabolites in urine | | *P* |
|---|---|---|---|
| 20 mg (i.v.) | 5-HT | $4813 \pm 1179$ $\mu$g | $<0{\cdot}02$ |
| | 5-HT-*O*-glucuronide | $466 \pm 167$ $\mu$g | $<0{\cdot}05$ |
| | 5-HIAA | $4223 \pm 924$ $\mu$g | $<0{\cdot}02$ |
| 200 mg (oral) | 5-HT | $4736 \pm 1472$ $\mu$g | $<0{\cdot}05$ |
| | 5-HT-*O*-glucuronide | $640 \pm 482$ $\mu$g | $>0{\cdot}2$ |
| | 5-HIAA | $10656 \pm 445$ $\mu$g | $<0{\cdot}001$ |

Increases in the excretion of 5-hydroxyindoles in 24 h urine specimens collected after administration of DL-5-HTP. Each value is a mean obtained from five wethers $\pm$ S.E.M.

In two animals 5-HT-*O*-glucuronide was excreted in increased amounts after the second dose of isocarboxazid and the raised level of excretion was maintained for 2 days after discontinuing the drug (Fig. 2); in the third sheep the excretion of 5-HT-*O*-glucuronide increased only after the administration of isocarboxazid had been stopped. Administration of the monoamine oxidase inhibitor increased the 5-HT content of the specimens by about 50% in two of the three animals. The increases in the excretion of 5-HT-*O*-glucuronide and 5-HT after administration of isocarboxazid were always substantially less than the reduction in urinary 5-HIAA.

*Effect of carbon tetrachloride on metabolites of endogenous 5-HT*

Four sheep were put in metabolism cages for 7 days and carbon tetrachloride (4 ml) was administered orally at the end of the third day of the experiment. The

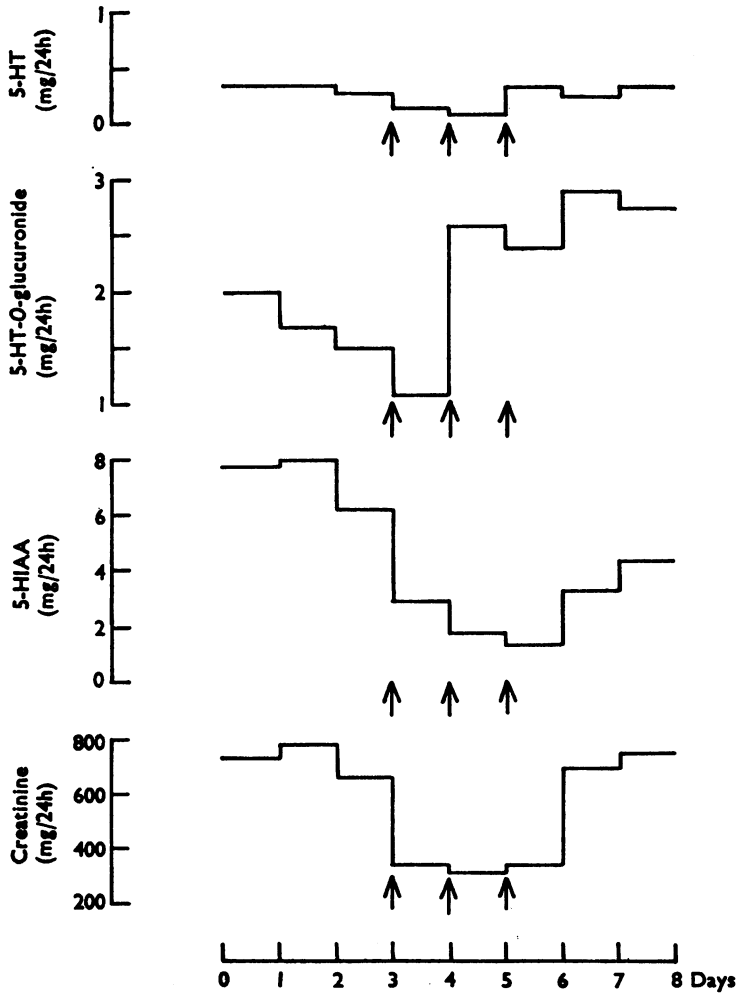


FIG. 2. Effect of isocarboxazid on the excretion of 5-hydroxyindolyl compounds in the urine of the sheep. Sheep, three, 33 kg. From above downwards: urinary 5-HT (mg/24 h); 5-HT-*O*-glucuronide (mg/24 h); 5-HIAA (mg/24 h) and creatinine (mg/24 h). At the arrows isocarboxazid (20 mg/kg) was adminstered orally on 3 successive days.

drug did not produce any symptoms in the animals or affect the volume and creatinine content of the urine specimens. The 5-HT-*O*-glucuronide content of the specimens was always reduced after administration of carbon tetrachloride (Fig. 3), being $49 \pm 2\%$ of the control content the first day after the drug and $42 \pm 6\%$, $51 \pm 7\%$ and $63 \pm 15\%$ on the second, third and fourth days, respectively. Carbon tetrachloride did not produce any consistent changes in the urinary excretion of 5-HT and 5-HIAA. In three of the animals the excretion of 5-HT was reduced for 24 h following administration of carbon tetrachloride, and in two animals there was some reduction in the 5-HIAA content of the specimens after administration of the drug.

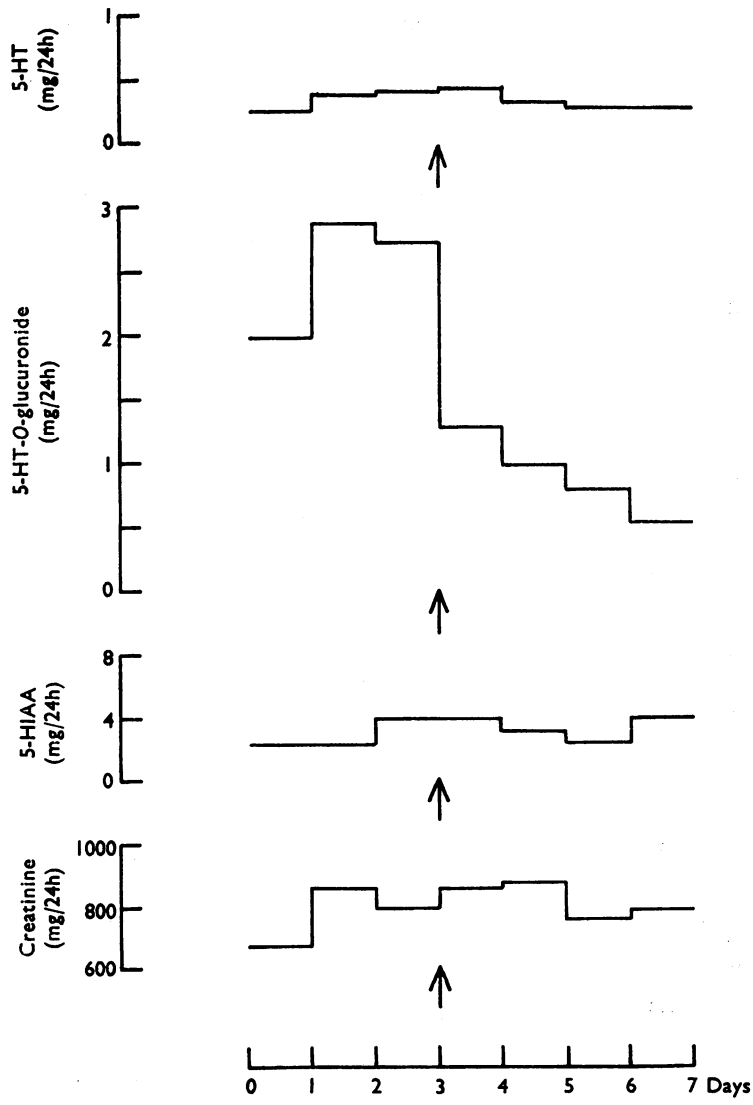


FIG. 3. Effect of carbon tetrachloride on the excretion of 5-hydroxyindolyl compounds in the urine of the sheep. Sheep, three, 33 kg. From above downwards: urinary 5-HT (mg/24 h); 5-HT-*O*-glucuronide (mg/24 h); 5-HIAA (mg/24 h) and creatinine (mg/24 h). At the arrow $CCl_4$ (4 ml) was administered orally.

**Discussion**

Intravenous injection of 5-HT in sheep did not produce a significant increase in the urinary excretion of the amine. 5-HT administered intravenously perfuses the lungs before the kidneys, which may account for the insignificant excretion of the injected amine as dog lung clears 5-HT from the circulation (Thomas & Vane, 1967). Most of the 5-HT was probably deaminated by monoamine oxidase as about 87% of the urinary metabolites of 5-HT injected in sheep was 5-HIAA. According to Thomas & Vane (1967) however, pretreatment with inhibitors of monoamine oxidase did not affect the removal of circulating 5-HT by dog lung, thus there may be species differences in the metabolism of 5-HT in the lung. Disregarding this possibility, however, an alternative explanation would be that after blockade of monoamine oxidase the lung removes 5-HT from the circulation by another metabolic route or by storage of the amine. In these experiments about 9% of the 5-HT administered intravenously was excreted as 5-HT-*O*-glucuronide, and it is possible that after blockade of monoamine oxidase the lungs metabolize 5-HT by conjugation with glucuronic acid.

5-HT absorbed from the gastro-intestinal tract perfuses the liver and the lungs before the kidneys and as both the liver and the lungs are highly effective in clearing 5-HT from the circulation (Thomas & Vane, 1967) the absence of a rise in urinary 5-HT after oral administration of the amine was expected. About 7% of the metabolites (derived from orally administered 5-HT) was 5-HT-*O*-glucuronide, thus the liver and the gastro-intestinal tract did not seem to be more effective than the lungs in conjugating administered 5-HT with glucuronic acid.

When DL-5-HTP was administered to sheep intravenously or orally, a large amount of 5-HT was excreted in the urine. Much of the administered DL-5-HTP seemed to escape decarboxylation in the gastro-intestinal tract, liver and lungs for 5-HT perfusing these organs would be metabolized before excretion. Thus the urinary 5-HT seems to have been formed from DL-5-HTP which had perfused the lungs before being decarboxylated, possibly in the kidney. The volume and creatinine content of the 24 h urine specimens were unaffected by DL-5-HTP administration, thus sheep kidneys were either insensitive to 5-HT or excreted the amine so rapidly that its effect was not apparent in a 24 h collection. The relative amounts of 5-HT-*O*-glucuronide and 5-HIAA in urine collected after administration of DL-5-HTP were similar to those present in specimens collected after 5-HT administration, thus 5-HT derived from administered DL-5-HTP was neither conjugated with glucuronic acid nor deaminated to a greater extent than administered 5-HT.

The relative proportions of the urinary metabolites of endogenous 5-HT differed from those of administered 5-HT or DL-5-HTP. Expressed as a percentage of the urinary 5-hydroxyindoles the metabolites of endogenous 5-HT consisted of 7% 5-HT, 20% 5-HT-*O*-glucuronide and 73% 5-HIAA. Thus conjugation with glucuronic acid seemed more prevalent in the metabolism of endogenous 5-HT than of administered 5-HT. This deduction does not take into account any effect of the large dose of 5-HT which was administered, however, and it may be that as more amine was presented for metabolism so a greater proportion was deaminated by monoamine oxidase.

The reduction in urinary creatinine and faeces after administration of isocarboxazid seem to have been due to the anorexia. The overall urinary excretion of

5-hydroxyindoles was also reduced after isocarboxazid, the decrease in urinary 5-HIAA being greater than the increases in 5-HT-*O*-glucuronide and 5-HT. Although the anorexia may have reduced the urinary excretion of 5-hydroxyindoles the reduction could equally well have been due to an inhibition in the synthesis of 5-HT after isocarboxazid since administration of iproniazid, another hydrazine derivative which inhibits monoamine oxidase, may reduce the synthesis of 5-HT by making vitamin $B_6$ unavailable as a cofactor (Davison, 1956; Bartlet, 1960). Isocarboxazid reduced the urinary excretion of 5-HIAA promptly but the increase in the excretion of 5-HT-*O*-glucuronide was preceded by a delay of 1–3 days. It seems unlikely that UDP-glucuronyl transferase acts as slowly as this; an inhibition in the synthesis of 5-HT after isocarboxazid would explain the delay but some redisposition or synthesis of the enzyme may have been required before additional 5-HT-*O*-glucuronide was produced.

Carbon tetrachloride reduces the glucuronide conjugating capacity of guinea-pig liver as measured by the formation of *p*-nitrophenol glucuronide in liver homogenate (Isselbacher & McCarthy, 1960). Although the urinary excretion of 5-HT-*O*-glucuronide was reduced after administration of carbon tetrachloride to sheep the excretion of unmetabolized 5-HT and 5-HIAA was not increased. This observation suggests that the unconjugated 5-HT was retained in the tissues of the sheep or, that the formation of endogenous 5-HT was inhibited after administration of carbon tetrachloride.

This work was supported by the Agricultural Research Council. I wish to thank Professor F. Alexander for his interest in this work, Dr. M. W. Parkes (Roche Products Ltd.) for the isocarboxazid and DL-5-hydroxytryptophan, Mr. R. Brown for making the faecal egg counts and Miss Margaret Pither and Mr. Ian Warwick for technical assistance.

## REFERENCES

BARTLET, A. L. (1960). The 5-hydroxytryptamine content of mouse brain and whole mice after treatment with some drugs affecting the central nervous system. *Br. J. Pharmac. Chemother.*, **15**, 140–146.

BARTLET, A. L. & GILBERT, F. M. (1970). Estimation of 5-hydroxyindolylacetic acid in sheep urine. *Clin. Chim. Acta*, **30**, 559–565.

BARTLET, A. L. & GILBERT, F. M. (1971). Estimation of urinary 5-hydroxytryptamine-*O*-glucuronide, a metabolite of endogenous 5-hydroxytryptamine in sheep. *Br. J. Pharmac.*, **41**, 530–539.

DAVISON, A. N. (1956). The mechanism of the inhibition of decarboxylases by isonicotinyl hydrazide. *Biochem. biophys. Acta*, **19**, 131–140.

ISSELBACHER, K. J. & MCCARTHY, E. A. (1960). Effect of carbon tetrachloride upon glucuronide formation by guinea-pig liver. *Proc. Soc. exp. Biol. Med.*, **103**, 819–822.

TAUSSKY, H. H. (1956). A procedure increasing the specificity of the Jaffe reaction for the determination of creatine and creatinine in urine and plasma. *Clin. Chim. Acta*, **1**, 210–224.

THOMAS, D. P. & VANE, J. R. (1967). 5-Hydroxytryptamine in the circulation of the dog. *Nature, Lond.*, **216**, 335–338.

(*Received February* 19, 1971)