REFERENCES

Aschaffenburg, R. & Drewry, T. (1955). *Nature, Lond.*, **176**, 218.
Glazer, A. N. & Smith, E. L. (1961). *J. biol. Chem.* **236**, 2942.
Gordon, W. B., Basch, J. J. & Kalan, E. B. (1961). *J. biol. Chem.* **236**, 2908.
Kalan, E. B., Gordon, W. G., Basch, J. J. & Townend, R. (1961). *Arch. Biochem. Biophys.* **96**, 376.
Ogston, A. G. & Tombs, M. P. (1957). *Biochem. J.* **66**, 399.
Piez, K. A., Davie, E. W., Folk, J. E. & Gladner, J. A. (1961). *J. biol. Chem.* **236**, 2912.
Tanford, C. & Nozaki, Y. (1959). *J. biol. Chem.* **234**, 2874.
Timasheff, S. W. & Townend, R. (1961). *J. Amer. chem. Soc.* **83**, 470.
Tombs, M. P. (1957). *Biochem. J.* **67**, 517.
Yanari, S. & Bovey, F. A. (1960). *J. biol. Chem.* **235**, 2818.

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Steroid Metabolism in the Cat

2. BILIARY AND URINARY EXCRETION OF METABOLITES OF [4-^{14}C]CORTICOSTERONE*

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When ^{14}C-labelled progesterone is administered to human subjects and animals radioactive metabolites appear in the urine and bile or faeces. In rodents metabolites in bile or faeces account for the greater part (about 70%) of the administered steroid and metabolites in urine for about 30% (Grady, Elliott, Doisy, Bocklage & Doisy, 1952; Shen, Elliott, Doisy & Doisy, 1954). In man about 30% of the administered dose is excreted in the bile of subjects with bile fistulae, but only about 13% in the faeces of intact subjects; most of the remainder of the dose can be accounted for as metabolites in urine (Sandberg & Slaunwhite, 1958). However, when [4-^{14}C]progesterone was administered intravenously to anaesthetized male cats virtually all of the excreted radioactivity appeared in the bile; in the six animals studied urine radioactivity did not exceed 1% of the dose (Taylor & Scratcherd, 1961). Since it is probable that progesterone is not secreted to any appreciable extent by male cats it seemed desirable to investigate the metabolism of a steroid hormone known to be secreted by this animal. Therefore [4-^{14}C]-corticosterone has been administered intravenously to anaesthetized male cats and the excretion of labelled metabolites in bile and urine has been determined. A preliminary account of this work has been published (Scratcherd & Taylor, 1961).

MATERIALS AND METHODS

4-^{14}C-labelled steroids. [4-^{14}C]Corticosterone (11β,21-dihydroxypregn-4-ene-3,20-dione; 44·4 μc/mg.) was obtained from the Endocrinology Study Section, U.S. Public Health Services, Bethesda, Md., U.S.A. Paper chromatography in benzene–methanol–water (2:1:1, by vol.) showed that 96–98% of the radioactivity had the same R_F as an authentic sample of corticosterone. The labelled steroid was dissolved in ethanol and stored at −15°; no detectable decrease in purity had occurred after 7 weeks. For the single-dose experiments the steroid in 0·4 ml. of ethanol was diluted with 0·6 ml. of 0·154 M-NaCl; for infusion experiments about 1 mg. of unlabelled corticosterone (kindly supplied from the M.R.C. Reference Steroid Collection by Professor W. Klyne) was added to the [4-^{14}C]corticosterone and dissolved in 2 ml. of ethanol and 18 ml. of 0·154 M-NaCl. [4-^{14}C]Progesterone (pregn-4-ene-3,20-dione) was obtained from The Radiochemical Centre, Amersham, Bucks.; solutions for injection were prepared as described by Taylor & Scratcherd (1961).

Operative procedure. Male cats were prepared as described by Taylor & Scratcherd (1961), except for a change in the method of collecting bile. A cannula was made by attaching polythene tubing to a stainless-steel tube (0·5 mm. internal diam. × 4·5 cm. long), which was passed up the common bile duct to about 3 cm. above its entrance to the duodenum and tied in place. The cystic duct was tied off as near as possible to its junction with the common duct. The volume of the cannula, estimated by weighing it empty and then full of water, was 0·12 ml.

All other materials and methods were as described by Taylor & Scratcherd (1961), except that water-saturated ether–ethyl acetate (3:1, v/v) was used for extraction.

RESULTS

Table 1 shows the excretion of radioactivity in the bile of cats after a single rapid injection (cats 1 and 2) and after a 45 min. infusion (cats 3 and 4) of [4-^{14}C]corticosterone. The volumes of bile collected and the concentration of total solids are also included to show that biliary function was main-

* Part 1: Taylor & Scratcherd (1961).

Table 1. *Excretion of radioactivity in bile of male cats after intravenous injection of* [4-^{14}C]*corticosterone*

A single dose was given to cats 1 and 2, and cats 3 and 4 were dosed by continuous infusion for 45 min.

Cat no.	Wt. (kg.)	Dose of steroid: Wt. (mg.)	Dose of steroid: 10^{-6} × Activity (counts/min.)	Collection period (min.)	Bile: Vol. (ml.)	Bile: Total solids: Wt. (mg.)	Bile: Total solids: Concn. (g./100 ml.)	Bile: Radioactivity: 10^{-6} × (total counts/min.)	Bile: Radioactivity: Percentage of dose	Distribution of radioactivity (% of total counts in sample) after successive hydrolyses: Neutral ether–ethyl acetate-soluble radioactivity: Non-conjugated	Glucuronide	Cold-acid-hydrolysed	Hot-acid-hydrolysed	Water-soluble	Total recovery after all hydrolyses (%)
1	3·0	0·11	4·87	60	1·6	23	1·4	2·893	59·3	0·1	16·4	6·1	40·9	27·1	90·6
				60	1·7	14	0·8	0·861	17·6	0	6·7	5·8	30·4	44·9	87·8
				60	1·3	10	0·7	0·295	6·1	0	3·4	6·5	21·4	60·0	91·3
				60	1·3	20	1·4	0·144	2·8	0	2·5	5·7	15·5	67·3	91·0
								Total	85·8						
2	3·4	0·11	4·87	20	0·2	6	2·8	0·043	0·9	0·1	20·2	4·6	27·9	51·1	103·9
				20	0·8	19	2·3	1·239	25·4	0·1	18·7	5·4	43·6	30·4	98·2
				20	0·7	18	2·3	1·039	21·3	0	6·9	4·5	32·9	54·4	98·7
				60	2·3	13	0·6	1·555	31·9	0	5·1	4·8	30·8	52·8	93·5
				60	2·2	16	0·7	0·392	8·0	0	2·4	2·5	12·8	74·0	91·7
				60	2·3	—	0·7	0·164	3·4	0	2·8	3·6	9·8	80·1	96·3
								Total	90·9						
3	3·2	1·20	4·86	60	2·1	29	1·4	2·660	54·1	0	8·3	4·5	19·5	60·0	92·3
				60	2·7	27	1·0	1·375	28·3	0	4·3	3·8	19·8	62·7	90·6
				60	2·5	32	1·3	0·313	6·4	0	3·5	5·4	20·2	69·0	98·1
				60	2·2	24	1·1	0·097	2·0	0	4·1	4·1	21·9	67·1	97·2
				60	1·7	14	0·8	0·040	0·9	0	4·0	5·0	22·4	67·5	98·9
								Total	91·7						
4	3·4	1·4	4·86	30	1·8	34	1·9	0·844	17·4	0	13·3	3·3	13·4	62·5	92·5
				30	1·7	31	1·8	1·724	35·4	0	11·4	4·1	12·5	67·0	96·0
				60	3·2	61	1·9	1·123	23·1	0	6·1	5·3	15·8	64·8	91·0
				60	3·0	57	1·9	0·285	5·5	0	5·3	4·2	9·8	75·6	94·3
				60	2·8	54	1·9	0·076	1·6	0	5·3	5·3	7·9	76·6	95·1
				60	2·5	48	1·9	0·035	0·8	0	5·4	4·0	8·6	74·4	92·5
								Total	83·8						

tained throughout the experiments. There was some variation in the volume of bile produced by different animals, ranging from 1·3 (cat 1) to 3·5 ml./hr. (cat 4), and in the total solids, which ranged from 0·6 to 2·8 g./100 ml. in cat 2. In animals from which bile was collected by intubation of the gall-bladder the corresponding values were 0·1–3·9 ml./hr. and 1·8–10·0 g. of total solids/100 ml. (Taylor & Scratcherd, 1961). This collection of bile by cannulation of the common duct and tying off the gall-bladder yields true liver bile and obviates any possibility of changes in the bile occurring in the gall-bladder.

Table 1 shows that excretion of radioactivity after administration of the steroid is very rapid and is virtually complete (83·8–91·7 % of the dose) in 4–5 hr. About half of the radioactivity is excreted in the first hour, even when the steroid is given as a continuous infusion over 45 min. When bile was collected for 20 min. periods over the first hour (cat 2) radioactivity was present in the first sample, even though only 0·2 ml. of bile was collected in that time. The second and third 20 min. samples each contained over 20 % of the administered radioactivity. A similarly rapid rate of excretion was shown by cat 4, which yielded 17·4 % in the first 30 min. and 35·4 % in the second; thus 52·8 % of the dose had been excreted within 15 min. of stopping the infusion. These results differ from those obtained in similar experiments with [4-^{14}C]progesterone, for after a single rapid injection of that steroid only about 66 % of the dose was recovered in the bile, and after infusion of steroid for 1 hr., only about 30 % in 4–6 hr. (Taylor & Scratcherd, 1961). Thus corticosterone metabolites are excreted in bile more rapidly and to a greater extent than are metabolites of progesterone. To ascertain whether this difference was due to the different technique used for the collection of bile, [4-^{14}C]progesterone was administered to two cats as a single, rapid injection or by continuous infusion over 45 min. Bile was collected in 60 min. periods: the amount of radioactivity excreted (as % of dose/hr.) after a single injection was 20·4, 16·9, 7·0, 4·5, 3·1 and 2·2 (total 54·1 % in 6 hr.), and, after infusion, 12·0, 14·6, 9·6, 6·1 and 4·2 (total 46·5 in 5 hr.). The difference in rate and total amount of excretion therefore appear to be a property of the steroids and not of the different technique of bile collection.

Table 1 also shows the distribution of excreted neutral metabolites after hydrolysis, the amount of radioactivity remaining water-soluble and the percentage recovery of radioactivity after application of the successive hydrolytic procedures.

Table 2 gives the corresponding results for the urine samples obtained from the same four cats. The amount of radioactivity in the urine after a

Table 2. *Excretion of radioactivity in urine of male cats after intravenous injection of* [4-^{14}C]*corticosterone*

Cat no.	Collection period (hr.)	Urine: Vol. (ml.)	Total solids: Wt. (g.)	Total solids: Conen. (g./100 ml.)	Radioactivity: 10^{-4} × (Total counts/min.)	Radioactivity: Percentage of dose	Distribution of radioactivity (% of total counts in sample) after successive hyrolyses: Neutral ether–ethyl acetate-soluble radioactivity: Non-conjugated	Glucuronide	Cold-acid-hydrolysed	Hot-acid-hydrolysed	Water-soluble	Total recovery after all hydrolyses (%)
1	2	3·9	0·84	21·6	4·09	0·8	0·2	2·9	2·4	5·1	80·0	91·6
	2	3·5	0·67	19·3	1·54	0·3						
					Total	1·1						
2	2	5·8	0·87	15·1	3·2	0·7	0·3	2·2	2·0	4·1	88·8	97·4
	2	2·4	0·49	20·4	1·2	0·2						
					Total	0·9						
3	2	4·3	1·03	24·0	2·4	0·5	0·3	2·5	3·2	3·9	81·0	90·9
	2	3·1	0·63	20·3	0·4	0·10						
	1	1·0	0·25	25·0	0·04	0·01						
					Total	0·61						
4	2	2·7	0·74	27·5	1·6	0·3	0·7	6·7	5·7	11·4	64·5	89·0
	2	2·2	0·56	25·6	0·9	0·1						
	1	1·2	0·29	23·9	0·2	0·04						
					Total	0·44						

single dose of steroid appears to be greater than after infusion, but only a small percentage of the dose was excreted by the kidney. Table 2 also shows the amounts of neutral radioactivity recovered after hydrolysis and the percentage recovery of radioactivity after the successive hydrolyses. Because of the small amounts of radioactivity in the urine, samples from each animal were combined for analysis.

DISCUSSION

Little is known about the metabolism of corticosteroids in the cat. Venous blood from the adrenals of cats treated with adrenocorticotrophic hormone contains corticosterone and hydrocortisone (17α,11β,21-trihydroxypregn-4-ene-3,20-dione) and sometimes 21-hydroxypregn-4-ene-3,11,20-trione and 11β-hydroxyandrost-4-ene-3,17-dione (11β-hydroxyandrostenedione) (Bush, 1953). The effects of hepatectomy and total abdominal evisceration on the metabolism of endogenous corticosteroids have been studied in the acutely adrenalectomized cat. The rate of disappearance of hydrocortisone and corticosterone from plasma from different sites showed that elimination of corticosteroids from the blood of anaesthetized cats was not confined to the liver and that most of the non-hepatic elimination occurred in the gastrointestinal tract. The plasma half-lives of hydrocortisone and corticosterone were not significantly different, but were much shorter than in human subjects; the plasma half-life of corticosterone was shorter in the cats than in rats (Bojesen & Egense, 1960).

The results now reported confirm those of Taylor & Scratcherd (1961) with [4-^{14}C]progesterone that, under the experimental conditions employed, the bile is the major route of excretion of the intravenously administered steroid hormones in the cat. At least 50 % of the corticosterone metabolites are excreted in 1 hr. by this route after rapid injection or infusion for 45 min. of steroid, and is virtually complete in 4–5 hr. whereas only about 1 % of the steroid appears as metabolites in urine. Intact human subjects excrete about 80 % of an intravenous dose of [4-^{14}C]corticosterone as metabolites in urine, whereas subjects with bile fistulae excrete 63–71 % of the dose in the urine and 20–30 % in the bile (Migeon, Sandberg, Paul & Samuels, 1956*b*). Dogs excrete 55 % of intravenously administered [4-^{14}C]corticosterone as metabolites in the urine and 20 % in the faeces; dogs with chronic bile fistulae excrete 30–65 % of the dose in the urine and 25–50 % in the bile (Willoughby, Chen & Freeman, 1959). Therefore in human subjects and dogs some enterohepatic recirculation of corticosterone metabolites occurs but is greater in human subjects than in dogs: some of the metabolites reabsorbed from the gastrointestinal tract subsequently appear in the urine. In contrast only small amounts of progesterone metabolites reabsorbed from the gastrointestinal tract of human subjects and rats appear in the urine, since the rates of excretion and amounts of urine radioactivity are similar in intact subjects and subjects with bile fistulae or in sham-operated animals and animals with fistulae (Grady *et al.* 1952; Shen *et al.* 1954; Sandberg & Slaunwhite, 1958). Normal rats, however, excrete metabolites of intravenously administered [4-^{14}C]hydrocortisone in the urine (31–36 % of the dose) and in the faeces (60–61 %), but animals with bile fistulae excrete only about 6 % of the dose as metabolites in the urine and 91 % in the bile (Hyde & Williams, 1957). In contrast with rats, however, intact human subjects excrete 76–83·3 % of intravenous hydrocortisone or cortisone as metabolites in the urine in 24 hr., and subjects with bile fistulae excrete 75·2–81·4 % (Migeon *et al.* 1956*a*; Peterson, Pierce, Wyngaarden, Bunim & Brodie, 1957). When progesterone or corticosterone is administered intravenously, intramuscularly or subcutaneously to the intact male cat only 0·6–4·0 % of the administered radioactivity appears in the urine in 3–7 days but 53–65·2 % can be detected in the faeces (Taylor & Scratcherd, 1962). Therefore there appears to be little or no reabsorption of metabolites of these hormones from the gastrointestinal tract of the cat, or if there is then the reabsorbed metabolites are excreted again by the liver and not by the kidney. Thus species differ in the routes by which they excrete steroid-hormone metabolites, and different steroids may be excreted by the same species by different routes.

Sandberg & Slaunwhite (1956) suggested that there is a relationship between binding of a steroid to serum albumin and the extent of its excretion in the bile; those steroids firmly bound (oestrogens, progesterone) are mainly excreted in the bile, whereas those weakly bound (hydrocortisone, 11β-hydroxyandrostenedione) do not appear in the bile to any great extent. This is not true for corticosterone in human subjects (Migeon *et al.* 1956*b*), cats (present investigation) or for hydrocortisone in rats (Hyde & Williams, 1957). Rodents and man metabolize steroids at different rates *in vivo*, and Berliner & Dougherty (1960) suggested that the rate of disposal of the steroid may be inversely proportional to the number of carbon and oxygen atoms in the molecule of the hormone; thus corticosterone ($C_{21}O_4$) has a shorter plasma half-life (30–35 min.) than hydrocortisone ($C_{21}O_5$) (40–50 min.). This generalization is not true for cats, because progesterone ($C_{21}O_2$) should be excreted more quickly than corticosterone.

Nature of the conjugates. The metabolites detected are of the same type as those found with pro-

gesterone in cats (Taylor & Scratcherd, 1961) and with corticosterone in man (Migeon *et al.* 1956*b*; Peterson & Pierce, 1960). The possible nature of these metabolites has already been discussed (Taylor & Scratcherd, 1961). The proportion of unconjugated metabolites in bile and urine samples was less after administration of corticosterone than after progesterone. With progesterone more metabolites were excreted as biliary glucuronides (maximum about 40 %) and as urinary glucuronides (maximum 7·4 %) than with corticosterone (16·4 % in bile and 6·7 % in urine). An even more marked difference was found with metabolites hydrolysed by cold acid. The maximum proportion for progesterone metabolites was 40 % in bile and 11·7 % in urine, and the respective values for corticosterone were 6·5 and 5·7 %. The proportions of hot-acid-hydrolysed metabolites of the two steroids in bile and urine were variable from animal to animal. However, a greater proportion of water-soluble metabolites was excreted in bile after corticosterone than after progesterone (Table 1), but there was little difference between the water-soluble metabolites of the two steroids in the urine (Table 2).

Variation in excretion of conjugates with time. Taylor & Scratcherd (1961) found that the proportions of progesterone metabolites extractable before and after the various hydrolytic procedures varied with time. The most consistent variation was in the biliary glucuronides, which showed progressive decrease in successive samples. The same trend can be seen to occur in the glucuronide fraction after administration of corticosterone (Table 1): the most striking examples of this were in cats 2 and 4 when bile samples were collected for less than 1 hr. Cold-acid-hydrolysed metabolites of progesterone increased in three animals and decreased in two, whereas these metabolites of corticosterone remained low and relatively constant. No consistent change occurred in hot-acid-hydrolysed metabolites of corticosterone; these decreased in cat 1, increased and decreased in cats 2 and 4 and remained virtually constant in cat 3 (Table 1). A similar variation in hot-acid-hydrolysed metabolites of progesterone was also observed, but in four out of five animals there was a definite increase in the proportion of metabolites remaining water-soluble after all forms of hydrolysis (Taylor & Scratcherd, 1961). In all animals given corticosterone there was a general increase in the proportion of water-soluble metabolites with time (Table 1).

Berliner, Leong, Cazes & Berliner (1962) found that isolated rat livers excreted corticosterone metabolites more rapidly than those of hydrocortisone, and that the proportion of glucuronides in successive bile samples decreased with time whereas cold-acid-hydrolysed metabolites remained fairly constant. The major part of the dose was excreted as metabolites that remained water-soluble after various forms of hydrolysis.

There are conflicting views about the mechanism of secretion of substances into bile. Substances may pass from the blood into the parenchymal cells and then be secreted into the bile canaliculi and thence to the bile ducts. Alternatively some constituents of bile, particularly bilirubin glucuronides, may be formed in parenchymal cells, pass back into the blood and then be secreted by the biliary epithelium and then into the bile ductules (Andrews, 1955, 1958; Heikel, Knight & Rimington, 1960). The rapid excretion of corticosterone metabolites into cat bile supports the theory of Andrews, particularly when the results in cat 2 (Table 1) are examined. The 'dead space' of the whole biliary tree is difficult to determine but the volume of the cannula was 0·12 ml. The larger proportion of steroid glucuronides in the earlier bile samples might also be explained on the theory that these glucuronides diffuse more readily from the liver cells into the blood and are more rapidly excreted by secretion across the biliary epithelium. Some glucuronides may also be secreted into bile via the parenchymal cells, or the other non-glucuronide metabolites may pass from the blood across the biliary epithelium. However, reabsorption of water may occur as the bile passes down the biliary tree, resulting in concentration of the bile, and it may be significant that the 0·2 ml. of bile collected in the first 20 min. from cat 2 had a relatively high concentration of total solids (2·8 g./100 ml.) (Table 1). But water-reabsorption was less in the present experiments, in which the gall-bladder was tied off, than in the previous investigation. Total bile solids ranged from 0·6 to 2·8 g./100 ml., whereas bile collected by intubation of the gall-bladder had 1·8–10·0 g. of total solids/100 ml. (Taylor & Scratcherd, 1961). Investigations on cats with bile fistulae (Taylor & Scratcherd, 1961; and present investigation) and with the intact animal (Taylor & Scratcherd, 1962) indicate that the cat may excrete steroid hormones almost entirely via the liver.

SUMMARY

1. [4-^{14}C]Corticosterone has been administered to anaesthetized male cats as a single injection or as a 45 min. infusion. Bile and urine were collected for periods of up to 5 hr. after the start of the injection, and radioactive metabolites were determined in the two fluids.

2. Excretion of the dose of corticosterone was almost complete (84·2–92·3 %) in 4–5 hr. Most of the radioactivity appeared in the bile and only a little (0·4–1·1 %) in the urine.

3. Bile and urine samples were hydrolysed by standard methods: metabolites excreted included unconjugated material, glucuronides and substances hydrolysed by cold and hot acid; the major part of the excreted radioactivity was present as substances remaining water-soluble after all forms of hydrolysis.

4. When samples of bile were collected over periods of 1 hr. or less the proportion of radioactivity excreted as glucuronide always decreased in successive samples whereas water-soluble metabolites generally increased. Substances hydrolysed by cold acid remained virtually constant whereas those hydrolysed by hot acid showed no consistent change.

5. The relationship of these results to similar studies of progesterone metabolism in the cat and of other steroids in man and animals is discussed.

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REFERENCES

Andrews, W. H. H. (1955). *Lancet*, ii, 166.
Andrews, W. H. H. (1958). In *Liver Function*, p. 241. Ed. by Brauer, R. W. Washington, D.C.: American Institute of Biological Sciences.
Berliner, D. L. & Dougherty, T. F. (1960). *Ann. N.Y. Acad. Sci.* **88**, 14.
Berliner, D. L., Leong, G. F., Cazes, D. M. & Berliner, M. L. (1962). *Amer. J. Physiol.* **202**, 420.
Bojesen, E. & Egense, J. (1960). *Acta Endocr., Kbh.*, **33**, 347.
Bush, I. E. (1953). *J. Endocr.* **9**, 95.
Grady, H. J., Elliott, W. H., Doisy, E. A., jun., Bocklage, B. C. & Doisy, E. A. (1952). *J. biol. Chem.* **195**, 755.
Heikel, T., Knight, B. C. & Rimington, C. (1960). *Proc. Roy. Soc.* B, **153**, 47.
Hyde, P. M. & Williams, R. H. (1957). *J. biol. Chem.* **227**, 1063.
Migeon, C. J., Sandberg, A. A., Decker, H. A., Smith, D. F., Paul, A. C. & Samuels, L. T. (1956*a*). *J. clin. Endocr.* **16**, 1137.
Migeon, C. J., Sandberg, A. A., Paul, A. C. & Samuels, L. T. (1956*b*). *J. clin. Endocr.* **16**, 1291.
Peterson, R. E. & Pierce, C. E. (1960). *J. clin. Invest.* **39**, 741.
Peterson, R. E., Pierce, C. E., Wyngaarden, J. B., Bunim, J. J. & Brodie, B. B. (1957). *J. clin. Invest.* **36**, 1301.
Sandberg, A. A. & Slaunwhite, W. R. (1956). *J. clin. Endocr.* **16**, 923.
Sandberg, A. A. & Slaunwhite, W. R. (1958). *J. clin. Endocr.* **18**, 253.
Scratcherd, T. & Taylor, W. (1961). *J. Physiol.* **159**, 83*P*.
Shen, N.-H. C., Elliott, W. H., Doisy, E. A., jun. & Doisy, E. A. (1954). *J. biol. Chem.* **208**, 133.
Taylor, W. & Scratcherd, T. (1961). *Biochem. J.* **81**, 398.
Taylor, W. & Scratcherd, T. (1962). *International Congress on Hormonal Steroids, Milan; Excerpta Medica*, no. **51**, p. 142.
Willoughby, H. W., Chen, C. & Freeman, S. (1959). *Endocrinology*, **65**, 539.

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The Metabolism of Ethanol in Rat-Liver Suspensions

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The work of Batelli & Stern (1910), in which minced horse liver was employed, gave only a very crude idea of the rate at which ethanol is metabolized, as the conditions used were not optimum. Leloir & Muñoz (1938) carried out a valuable series of experiments on the metabolism of liver slices in the presence of ethanol. The metabolism of ethanol by liver preparations has not been extensively studied, mainly because this is a relatively slow process, and the analytical methods available to the earlier workers lacked the precision necessary to measure the small changes in ethanol concentration that occur in the limited periods during which liver slices and suspensions are stable. Very accurate determination of the ethanol concentration is essential if the rate of metabolism is to be measured reliably within a period of 1 or 2 hr. The enzymic method for ethanol determination (Horecker & Kornberg, 1948; Bonnichsen & Theorell, 1951; Bücher & Redetzki, 1951) is sufficiently accurate and specific for this purpose.

The aim of the present work was to study the kinetics of ethanol metabolism in liver suspensions, to examine the possible role of catalase and to determine the influence of a number of substances which have been claimed to accelerate the metabolism *in vivo*. A preliminary account of the work has been given (Svendsen & Lundquist, 1960).