The brain concentrations of diazepam and its N-desmethyl metabolites were measured with a sensitive gas chromatographic method taking advantage of an electron capture detector. Table 1 shows that the rate of disappearance of diazepam from brain is similar in rats and in mice.

TABLE 1. *Anticonvulsant effect and levels of diazepam in brain after administration of diazepam (5 mg/kg intravenously)*

Rat				Mouse			
Time after diazepam	Diazepam (μg/g)	N-Des-methylated metabolites (μg/g)	Anti-metrazol effect*	Time after diazepam	Diazepam (μg/g)	N-Des-methylated metabolites (μg/g)	Anti-metrazol effect*
1 min	3·270 ±0·050	0·101 ±0·007	100	1 min	3·407 ±0·070	–	100
30 min	0·600 ±0·010	0·065 ±0·021	100	30 min	0·495 ±0·023	0·781 ±0·070	100
60 min	0·305 ±0·070	0·051 ±0·037	100	60 min	0·392 ±0·041	0·490 ±0·022	100
2 hr	0·020 ±0·004	-	20				
3 hr	-	-	0				
5 hr	-	-	0	5 hr	n.d	0·732 ±0·061	100
10 hr	-	-	0	10 hr	-	0·390 ±0·033	100
15 hr	-	-	0	15 hr	-	n.d.	100
20 hr	-	-	0	20 hr	-	n.d.	80
24 hr	-	-	0	24 hr	-	n.d.	60

* Percentage of animals protected from the convulsions induced by metrazol (100 mg/kg intraperitoneally).
n.d., Not detectable.

In contrast, while the N-desmethyl metabolites are present in mice brain for about 15 hr, only traces were found in the brain of rats and these were detected for only 2 hr. Studies on the toxicity and antimetrazol activity of diazepam and the metabolites N-desmethyldiazepam and oxazepam, indicate that the N-desmethyl metabolites of diazepam are less toxic than diazepam but they exert an anticonvulsant activity similar to the parent compound. It is therefore suggested that the different effect of diazepam in rats and mice may be related to a different rate of formation or brain accumulation of the N-desmethylated metabolites.

This study was supported by the National Institutes of Health, Bethesda, Maryland, U.S.A., Contract DHEW/PHS/NIH/PH 43-67-83.

Metabolism of prostaglandins by the rat isolated liver

W. DAWSON*†, P. W. RAMWELL and JANE SHAW, *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, U.S.A.*

Prostaglandins are rapidly removed from the circulating blood (Ferreira & Vane, 1967), yet little is known either of the mechanism of uptake by the tissues, or of the subsequent metabolism (Änggard & Samuelsson, 1966). Following systemic injection of tritium labelled prostaglandin E_1 (PGE_1) to rats, rapid uptake of labelled

material by the lungs, kidneys and also by the liver has been reported (Samuelsson, 1964). We have therefore used the blood perfused rat isolated liver (Abraham & Dawson, 1967) to study the uptake and metabolism of prostaglandins, and also to monitor continuously the haemodynamic, biochemical and blood gas changes in arterial and venous blood during PGE_1 infusion (0.001–1 μg/min).

1–^{14}C PGE_1 (40 μg) was injected into the portal vein; 15×1 min samples of bile and blood from the vena cava were collected. The blood was run directly into 95% ethanol and the free fatty acids and prostaglandins were extracted (Shaw & Ramwell, 1968); the bile samples were assayed directly for determination of radioactivity; on termination of the experiment the liver was frozen before extraction of the prostaglandins. Following a single injection of PGE_1, evidence for a rapid uptake by the liver was obtained, for only 3–9% of the radioactivity could be detected in the hepatic venous blood. Peak output of labelled material, which was identified with products less polar than PGE_1, occurred at 1–3 min, and a secondary rise (0.05–0.7% of the injected radioactivity) was evident towards the termination of the experiment. Little radioactivity was detected in the bile (0.3–0.8%, peak output at 3–4 min). Of the remaining radioactivity only 15–20% was detected in the liver, 96% of which was not associated with PGE_1, but with less polar products

In subsequent experiments 1–^{14}C PGE_1 was allowed to recirculate through the liver preparation for 2 hr in a closed system with a CO_2 trap inserted. As previously, only a small fraction of the injected radioactivity was detected in the bile, and blood samples removed at 15 min intervals were again found to contain labelled material less polar than the prostaglandins. However, 15–20% of the injected radioactivity was detected as exchanged $^{14}CO_2$, which reached a peak at 10–15 min, and suggested that β oxidation of the C1–8 side chain had occurred. The radioactivity remaining in the liver (25–30%) was identified by thin-layer and gas-liquid chromatography with lecithin and C16–22 fatty acids, and probably arose from incorporation of labelled fragments from prostaglandin metabolism.

In contrast, following recirculation of 5–6^{3}H PGE_1, 19–30% (peak at 10 min) of the injected radioactivity was detected in the bile; by extrapolation from the 1–^{14}C PGE_1 studies, it can be deduced that the label was associated with a compound differing from PGE_1 by at least one methylene group; retention of the cyclopentane ring is inferred by finding a similar biliary secretion (20–28%) of $PGF_{1\alpha}$ labelled at the C9 position within the ring.

These results indicate that oxidation of prostaglandins within the liver, followed by biliary secretion, may be a significant pathway for prostaglandin metabolism.

W. D. is most grateful to the Wellcome Trust for a Travel Grant.

† Visiting scientist, present address: British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey, England.

REFERENCES

ABRAHAM, R. & DAWSON, W. (1967). Criteria for the physiological perfusion of the isolated rat liver. *J. Physiol., Lond.*, **192**, 92*P*.

ÄNGGARD, E. & SAMUELSSON, B. (1966). Metabolites of prostaglandins and their biological properties. *Memoirs of the Society for Endocrinology*, ed. Pickles, V. R. & Fitzpatrick, J. R., No. 14, pp. 107–117. London: Cambridge University Press.

FERREIRA, S. H. & VANE, J. R. (1967). Prostaglandins: their disappearance from and release into the circulation. *Nature, Lond.*, **216**, 868–873.

SAMUELSSON, B. (1964). Synthesis of tritium-labelled PGE_1 and studies on its distribution and excretion in the rat. *J. biol. Chem.*, **239**, 4091–4096.

SHAW, J. E. & RAMWELL, P. W. (1968). Release of prostaglandin from rat epididymal fat pad on nervous and hormonal stimulation. *J. biol. Chem.*, **243**, 1498–1503.