4-fold by mid-pregnancy to 0.52. In rabbits, the rates of steroid transformation were stimulated 1 to 2-fold by PB only in early pregnancy, and in contrast to *N*-demethylation, they responded to this drug neither in non-pregnant animals nor at mid-pregnancy.

In the present study with rats, the hepatic microsomal content of cytochrome P-450 and the enzymic rates of *N*-demethylation of aminopyrine and 7α-hydroxylation of DHA were significantly increased early in pregnancy over non-pregnant levels, but by the end of pregnancy these values had decreased significantly below those of non-pregnant females. This correlation between drug- and steroid-metabolizing enzymes has previously been noted in non-pregnant rats [4, 10], and now appears in pregnancy, either with or without PB treatment. Previous workers have demonstrated that pregnancy in rats suppresses several drug-metabolizing enzyme activities, and that cytochrome P-450 content was slightly increased in comparison to non-pregnant animals [16, 17]. In rabbits, only the *N*-demethylase activity follows the changes in cytochrome P-450. All of the steroid hydroxylase activities are depressed by PB treatment of mid-pregnant rabbits. We find no published reports of steroid hydroxylase activities in the liver of rats or rabbits during pregnancy.

It is clear from these and other studies that pregnancy and species differences profoundly influence maternal hepatic steroid- and drug-metabolizing activities and their selective responses to PB in rats and rabbits. Nevertheless, gross inspection of the embryos and reproductive tracts of PB-treated animals revealed no abnormalities, and the mothers appeared healthy. An activating effect of antenatal PB therapy on bilirubin conjugation by human newborn has been observed repeatedly, although little attention has been given to its effects on maternal metabolism (see Ref. 18 for a review). Preliminary observations (unpublished) in human pregnancies complicated by erythroblastosis indicate that the major steroid biosynthetic pathway leading to the maternal excretion of estriol is not altered by antenatal PB therapy.

Acknowledgements—The valuable suggestions by Mont R. Juchau, Ph.D., Department of Pharmacology, during the preparation of this manuscript are appreciated. The technical assistance of Ms. Mary Waltman, Kimiko Fukushima and Robert Fallis, Ph.D., and the support of the Ford and The Lalor Foundations, and PHS Grant HD 03825 are gratefully acknowledged. The typing of the manuscript by Mrs. Ann Dow is also greatly appreciated.

Department of Obstetrics and Gynecology, TORU TABEI
University of Washington, W. LEROY HEINRICHS
School of Medicine,
Seattle, Wash. 98195, U.S.A.

REFERENCES

1. A. H. Conney, *Pharmac. Rev.* **19,** 317 (1967).
2. T. Tabei and W. L. Heinrichs, *Gynec. Invest.* **4,** 229 (1973).
3. T. Tabei and W. L. Heinrichs, *Endocrinology* **94,** 97 (1974).
4. W. L. Heinrichs and A. Colás, *Biochemistry* **7,** 2273 (1968).
5. A. Colás, D. Gregonis and N. Moir, *Endocrinology* **84,** 165 (1969).
6. M. Jacobson and R. Kuntzman, *Steroids* **13,** 326 (1969).
7. W. Levin and R. Kuntzman, *J. biol. Chem.* **244,** 3671 (1969).
8. A. H. Conney and A. Klutch, *J. biol. Chem.* **238,** 1161 (1963).
9. T. Fujita, D. W. Shoeman and G. J. Mannering, *J. biol. Chem.* **248,** 2192 (1973).
10. T. Tabei K. Fukushima and W. L. Heinrichs, *Endocrinology* **96,** 815 (1975).
11. A. H. Conney and R. Kuntzman, in *Handbuch der Experimentellen Pharmalcologie* (Eds. B. B. Brodie and J. R. Gillette), Vol. XXVII, Part II, p. 403. Springer, Berlin (1971).
12. W. L. Heinrichs, H. Feder and A. Colás, *Steroids* **9,** 23 (1967).
13. S. Orrenius, *J. Cell. Biol.* **26,** 713 (1965).
14. T. Omura and R. Sato, *J. biol. Chem.* **239,** 2370 (1964).
15. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177,** 751 (1944).
16. M. G. Neale and D. V. Parke, *Biochem. Pharmac.* **22,** 1451 (1973).
17. E. Schlede and R. Borowski, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **281,** 341 (1974).
18. J. W. Reynolds, *Clin. Obstet. Gynec.* **17,** 95 (1974).

Biochemical Pharmacology, Vol. 25, pp. 2101–2102, Pergamon Press, 1976. Printed in Great Britain.

Effect of ingestion of *Lantana camara* L. on bile formation in sheep

(*Received* 17 *March* 1976; *accepted* 7 *April* 1976)

The triterpene acids lantadene A and icterogenin have been shown in rabbits to inhibit hepatic transport of porphyrins, bile pigments and bromsulphthalein [1], but little information is available on their effects on other transport processes which may be involved in bile formation. Some observations have been made on pathological changes in the sheep's liver and biliary tract after ingestion of the plant *Lantana camara*, an important source of lantadene A [2, 3, 4]. These pathological changes include jaundice, gall bladder distension, and proliferation of bile ductules [3]. In sheep, the major stimulants to bile secretion are the bile acid, taurocholic acid, and the hormone secretin [5, 6, 7]. These stimulants appear to act at different sites: the bile acid at the canaliculi, and secretin at the ductules. For this reason, they were used to analyse the effects of lantana on bile formation.

These studies were made on four crossbred sheep which had been operated on to remove the gall bladder, and to

place a polyvinylchloride cannula in the proximal segment of the common bile duct to enable bile to be collected. The sheep were allowed to recover after the operations, and were placed in metabolism cages.

Experiments were done before, and one day after, lantana was given. Each sheep was starved for 24 hr then two doses, each of 100 g of the air-dried leaf, were given by stomach tube three hours apart. The concentration of total bilirubin in the serum increased from 4.1 ± 0.03 μmole/l before the lantana was given, to 47.7 ± 1.81 μmole/l one day later; this was taken to indicate that intoxication had occurred.

In the experiments, taurochloric acid (T-0750; Sigma Chemical Company, St. Louis Mo.) in a concentration of 97 m-mole/l was infused through a jugular cannula at 0.22 ml/min. Samples of bile were collected immediately before the infusion and after 60 min of infusion, then secretin (Karolinska Institutet, Stockholm) was infused into the jugular vein at 0.44 clinical units/min for a 15-min preliminary period, and while bile samples were collected. The secretin and taurocholic acid infusions were then stopped, and a period of 45 min allowed for the effect of the secretin to abate [8]. In the next part of the experiment, taurocholic acid was infused at a rate similar to the maximum transport capability of the sheep liver [5]: a solution of 97 m-mole/l was infused at 0.88 ml/min, and was continued while bile samples were collected.

In each case, bile samples were collected for two consecutive periods, each of 10 min. The bile was collected under paraffin, and the volume estimated from the net weight. Samples were analysed for total bile salts [9] and bicarbonate [10]. The effects of the taurocholic acid and of the secretin were estimated from the differences between values in the samples collected before the infusion of that substance, and values in samples collected during that infusion. Analysis of variance was used to estimate the effect of lantana administration on the response to taurocholic acid, and to secretin.

One day after the lantana was given the output of bile acids in response to infusions of taurocholic acid at 20 μmole/min had decreased almost to zero:

before lantana: 26 ± 3.6 μmole/min,
after lantana: 0.3 ± 0.16 μmole/min

This effect of lantana on bile secretion was reflected in the bile flow. The mean basal bile flow was 0.17 ± 0.032 ml/min before lantana was given, and the flow increased by 0.36 ± 0.033 ml/min during infusion of taurocholic acid. However, the response was only 0.12 ± 0.030 ml/min after ingestion of lantana ($P < 0.001$). There was no basal bile flow in three sheep after lantana was given and the fourth sheep had a basal flow of 0.20 ml/min.

The effect of lantana on the response to infusion of taurocholic acid at 80 μmole/min was profound, but it did differ somewhat between sheep. In two of the sheep, the ability of the liver to transport this level of bile acid appeared to have been lost: the rate of secretion of bile acid did not increase at all during the infusion. In the other two sheep, bile acid secretion decreased:

sheep 1: before lantana 46 μmole/min; after lantana 32 μmole/min.
sheep 2: before lantana 62 μmole/min; after lantana 14 μmole/min.

The results of these experiments provide evidence that the toxic principle of *Lantana camara*, lantadene A, inhibits active secretion of bile acids into canaliculi. The precise mechanism of this inhibition is not clear, nor is it clear how this substance inhibits the transport of bromsulphthalein or bile pigments [2, 4]. However the observations of Goldfischer *et al.* [11] and Gopinath and Ford [4] that the activity of adenosine triphosphatase at the canaliculi is low after icterogenin or lantana, could indicate an effect on the availability of energy for active transport into the canaliculi. Although the responses to infusions of taurocholic acid were decreased in sheep after ingestion of lantana, the response to secretin showed no signs of diminution. In fact, the response in bile flow was enhanced: it was 0.26 ± 0.048 ml/min initially, and 0.44 ± 0.082 ml/min ($P < 0.025$) after the plant was given. The output of bicarbonate in response to infusion of secretin remained unchanged: it was 20 ± 2.5 μmole/min before, and 18 ± 3.2 μmole/min after lantana was given. This observation may be taken to indicate that lantadene A does not exert a toxic action on the ductules; indeed an increase in the function of ductule cells may occur. An increased biliary bicarbonate response to secretin has been described in human patients with cholestasis [12].

It is not clear whether the increased response to secretin is due to an increase in the sensitivity of individual cells, or to an increase in the number of ductular cells. Proliferation of bile ductules does occur during the first week after ingestion of lantana [4], but it is not likely that the number of ductular cells would have increased substantially during the one-day period used in the current study [see 3].

Acknowledgements—This work was supported by The Australian Meat Research Committee, and facilities were provided by the University Department of Physiology. Thanks are due to Mrs. E. Vasey and Mr. K. R. Smith for technical help, and to Mr. H. Thompson and his staff for bilirubin estimations.

Veterinary School, University of Queensland, St. Lucia, Queensland, Australia

MICHAEL A. PASS
ALAN A. SEAWRIGHT
TREVOR HEATH

REFERENCES

1. T. Heikel, B. C. Knight, C. Rimington, H. D. Ritchie and E. J. Williams, *Proc. R. Soc. B.* **153**, 47 (1960).
2. A. A. Seawright, *Aust. vet. J.* **39**, 340 (1963).
3. A. A. Seawright, *Path. vet.* **1**, 504 (1964).
4. C. Gopinath and E. J. H. Ford, *J. Path.* **99**, 75 (1969).
5. T. J. Heath, I. W. Caple and P. M. Redding, *Q. J. exp. Physiol.* **55**, 93 (1970).
6. T. J. Heath, *Q. J. exp. Physiol.* **55**, 301 (1970).
7. I. Caple and T. Heath, *Aust. J. biol. Sci.* **25**, 155 (1972).
8. I. W. Caple, Thesis, University of New South Wales (1972).
9. J. L. Irvin, C. G. Johnston and J. J. Kopala, *J. biol. Chem.* **153**, 439 (1944).
10. M. A. Segal, *Am. J. clin. Path.* **25**, 1212 (1955).
11. S. Goldfischer, I. M. Arias, E. Essner and A. B. Novikoff, *J. exp. Med.* **115**, 467 (1962).
12. Ch. Bode, D. Löber, H. Goebell, W. Dolle and K. Körner, *Acta hepato-gastroenterol.* **19**, 440 (1972).