the indole alkaloids of higher plants. The number of these indole alkaloids known has increased rapidly over the last few years, and over 600 are now known. A tryptamine residue appears almost invariably and, in the few cases examined by tracer methods, this residue has been found to be derived in the expected way from tryptophan. The remaining nine or ten skeletal carbon atoms appear in what at first sight seems a bewildering variety of different arrangements, but closer inspection allows three main groups to be discerned. The paper will be concerned first with the terpenoid origin of these three non-tryptamine units and secondly with the way in which the tryptamine portion of the final products is built on to the terpenoid portion. In each of these sections two important phases are

apparent. The initial phase involves determination of those substances that are used as the building blocks. With this achieved, the second phase can be initiated; here the intermediates on the pathway are identified and this in turn leads to a study of the mechanism of each step in the biosynthetic sequence. At present a most exciting stage has been reached where hypothesis, tracer experiment, structure determination, and isolation work can be combined in a very powerful way. All these aspects will be illustrated. Extensive use is made of ^{14}C labelling in the initial phase and of multiple labelling with ^3H and ^{14}C in the second phase. The substances used in this work include mevalonate, geraniol, merol, linalool, loganin, secologanin, vincoside and, of course, the indole alkaloids.

COMMUNICATIONS

Studies on the Relationship Between Molecular Structure and the Biliary Excretion of Organic Compounds

By P. C. HIROM, P. MILLBURN, R. L. SMITH and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London W.2*)

A diversity of organic compounds are excreted in the bile (Smith, 1966). Molecular weight and polarity are important factors in determining the extent to which a compound undergoes biliary elimination (Abou-El-Makarem, Millburn, Smith & Williams, 1967*a,b*; Millburn, Smith & Williams, 1967*a,b*). However, although certain structural features of a molecule probably influence its biliary excretion (Ryan & Wright, 1962; Williams, Millburn & Smith, 1965; Smith & Williams, 1966; Hearse, Powell, Olavesen & Dodgson, 1969), the relationship between molecular structure and biliary elimination remains obscure. Therefore, the biliary excretion of about 25 N^4 -acyl sulphonamides of closely related structures was examined. The compounds (100 $\mu\text{moles/kg}$. in 0.2 ml. of dimethyl sulphoxide) were injected intraperitoneally into biliary-cannulated female Wistar albino rats, and the 24 hr. bile was collected and analysed (Millburn *et al.* 1967*b*). They were excreted mainly unchanged in the bile.

The biliary excretion, expressed as a percentage of the dose, of the N^4 -succinyl derivatives of sulphanilamide, sulphacetamide and sulphathiazole (mol.wt. 272, 314 and 355 respectively) was 5, 4 and 54% respectively. The corresponding N^4 -phthalyl derivatives (mol.wt. 319, 362 and 403 respectively) had biliary excretions of 7, 2 and 19%. Thus the presence of a thiazole ring in the N^1 -position of

the above sulphonamides enhances their biliary elimination.

The effect of modifying the N^4 -substituent was investigated with a series of sulphathiazole derivatives. The excretion in the bile of hexahydrophthalyl-, 3-carboxy-2-naphthoyl-, tetrachlorophthalyl- and tetrabromophthalyl-sulphathiazole (mol.wt. 409, 453, 541 and 719 respectively) was about 60% of the dose irrespective of molecular weight. Thus, replacing the benzene ring of the phthalyl portion of phthalylsulphathiazole by a cyclohexane, a naphthalene or a halogenated benzene ring increased biliary excretion threefold.

ω -Carboxyacylsulphathiazoles containing as the N^4 group $\text{HO}_2\text{C} \cdot [\text{CH}_2]_n \cdot \text{CO}-$ with $n=0-8$ (mol.wt. 327-439) were also studied. When $n=0$ or 1, the biliary excretion was 15-20% of the dose; when $n=2$ or 4-8, the excretion was 40-50%; but when $n=3$, the high value of 74% was obtained.

These results support the hypothesis that polar molecules with molecular weights of at least 325 ± 50 are excreted in the bile of rats to the extent of 10% or more of the dose (Millburn *et al.* 1967*a*). However, above the minimum requirement there appears to be no close correlation between molecular weight and extent of biliary excretion in this series of compounds. These experiments also show that modifying the structure of a molecule can markedly alter its affinity for hepatobiliary elimination. The results will be further discussed in relation to molecular structure and physicochemical properties.

This work was supported by a grant from May and Baker Ltd.

Abou-El-Makarem, M. M., Millburn, P., Smith, R. L. & Williams, R. T. (1967*a*). *Biochem. J.* **105**, 1269.

- Abou-El-Makarem, M. M., Millburn, P., Smith, R. L. & Williams, R. T. (1967b). *Biochem. J.* **105**, 1289.
- Hearse, D. J., Powell, G. M., Olavesen, A. H. & Dodgson, K. S. (1969). *Biochem. Pharmacol.* **18**, 181.
- Millburn, P., Smith, R. L. & Williams, R. T. (1967a). *Biochem. J.* **105**, 1275.
- Millburn, P., Smith, R. L. & Williams, R. T. (1967b). *Biochem. J.* **105**, 1283.
- Ryan, A. J. & Wright, S. E. (1962). *Nature, Lond.*, **195**, 1009.
- Smith, R. L. (1966). In *Progress in Drug Research*, vol. 9, p. 299. Ed. by Jucker, E. Basle and Stuttgart: Birkhäuser Verlag.
- Smith, R. L. & Williams, R. T. (1966). In *Glucuronic Acid Free and Combined*, p. 457. Ed. by Dutton, G. J. New York and London: Academic Press Inc.
- Williams, R. T., Millburn, P. & Smith, R. L. (1965). *Ann. N.Y. Acad. Sci.*, **123**, 110.

Influence of Biliary Constituents in an Acid Medium on the Micellar Solubilization of Unesterified Fatty Acids of Sheep Duodenal Digesta

By AILEEN M. SCOTT and A. K. LOUGH. (Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB)

In sheep, the major lipid fraction of postruminal digesta comprises unesterified fatty acids (mainly stearic acid, palmitic acid and octadecenoic acid) (see Garton, 1967); these acids are associated with particulate matter (Lennox, Lough & Garton, 1965). Though Hofmann (1966) suggested that partial ionization of fatty acids is of importance for their micellar solubilization in the intestinal lumen, Lennox & Garton (1968) found that unesterified fatty acids were absorbed from the upper jejunum of the sheep where the luminal pH was in the range 2.8–5.0, indicating that un-ionized fatty acids were probably present in a soluble form. Accordingly the effect of biliary components on the solubilization of fatty acids under acid conditions was investigated with upper-duodenal digesta obtained from a cannulated grass-fed sheep.

Duodenal contents (8ml.) were shaken at 37° and at pH 4.0 (sodium hydrogen carbonate was added, if necessary) for periods of up to 24 hr. with sheep gall-bladder bile (2ml.) or aqueous solutions (2ml.) of bile salt (as taurocholate) with and without bile lecithin or bile lysolecithin or both in amounts corresponding to those present in the whole bile which (per 100ml.) contained 7.8g. of bile salt, 885mg. of lecithin and 255mg. of lysolecithin. The amount and composition of the unesterified fatty acids were determined in the original digesta and in the micellar and solid phases after incubation.

With whole bile, maximal solubilization was reached within 90min. giving a net transfer of

5.6mg. of fatty acid to the micellar phase from 9.1mg. originally associated with particulate matter; a preferential solubilization of stearic acid was observed.

In relation to the amount of fatty acids solubilized in the presence of whole bile the proportion of fatty acid taken into solution when taurocholate alone was added to duodenal digesta was about 30% while the addition of lecithin increased this to 70%. Corresponding values for lysolecithin and for lecithin plus lysolecithin were 50 and 80% respectively. Under all these conditions, solubilization of fatty acids was non-selective though maximal solubilization was reached apparently no less rapidly than with whole bile.

This solubilization of un-ionized fatty acids resembles that of the insoluble non-swelling amphiphile cholesterol in a bile salt–lecithin–water milieu (Small, 1968).

- Garton, G. A. (1967). *Wld Rev. Nutr. Diet.* **7**, 225.
- Hofmann, A. F. (1966). *Gastroenterology*, **50**, 56.
- Lennox, A. M. & Garton, G. A. (1968). *Brit. J. Nutr.* **22**, 247.
- Lennox, A. M., Lough, A. K. & Garton, G. A. (1965). *Biochem. J.* **96**, 27P.
- Small, D. M. (1968). *J. Amer. Oil Chem. Soc.* **45**, 108.

Electrophoretic Study of the Small Subunits of the Myosin Molecule

By W. T. PERRIE, S. V. PERRY and D. STONE. (Department of Biochemistry, University of Birmingham)

On polyacrylamide-gel electrophoresis of myosin from adult rabbit white muscle in the presence of 8M-urea and 10mM-mercaptoethanol, four main bands were obtained. Often, even with very fresh myosin, two additional faint bands were apparent, but these appeared to be modifications of the major bands, for on storage further bands appeared, particularly at alkaline pH values and in the absence of thiol compounds. A considerable proportion of the applied sample remained at the origin when the gel was made up in 8M- or 12M-urea.

In myosin from rabbit heart muscle, the fastest moving band was completely absent. Myosin from rabbit red muscle gave a pattern intermediate between those obtained with white- and cardiac-muscle myosin, with the fastest band less dense than the slower ones. Locker & Hagyard (1968) have reported similar results using acetylated myosins. Myosin from foetal and 1-day-old rabbits gave a much more complex picture than that from adults. This was probably due to the difficulties in removing impurities from these preparations, but a band pattern similar to that of red muscle could be identified. Four-band patterns very similar to those