

Both *i*-urobilin and *d*-urobilin are readily dehydrogenated by ferric chloride to give biliviolinoid pigments, but with *d*-urobilin this change can be effected even in a reducing environment (lithium aluminium hydride in tetrahydrofuran). This observation supports the above structure for *d*-urobilin since the conjugated system of double bonds shown in ring *D* would impart considerable tautomeric lability to the hydrogen atom attached to the α -carbon atom, and simple isomerism (involving neither oxidation nor reduction) to a biliviolin is therefore formally possible.

C. H. GRAY
D. C. NICHOLSON

Department of Chemical Pathology,
King's College Hospital Medical School,
London, S.E.5.
April 8.

¹ Lowry, P. T., Ziegler, N. R., and Watson, C. J., *Bull. Univ. Minn. Hosp. and Med. Found.*, **24**, 166 (1952). Watson, C. J., and Lowry, P. T., *J. Biol. Chem.*, **218**, 633 (1956).

² Plieninger, H., and Decker, M., *Liebigs Annalen*, **598**, 198 (1956).

Inhibition of Adrenal 11 β -Hydroxylation by Ions

HYDROXYLATION of the 11-position of the steroid nucleus is an essential step in the biosynthesis of the physiologically active adrenal corticoids, cortisol and aldosterone. Previous investigations¹⁻³ have shown that the reaction is rather involved, requiring the participation of a reducing agent (reduced triphosphopyridine nucleotide), molecular oxygen and several enzymes.

In the present study, the latter were extracted from acetone powders prepared from bovine or porcine adrenal mitochondria, and hydroxylating activity was assayed fluorimetrically, as described earlier³. During attempts to fractionate the adrenal extracts with ammonium sulphate and other salts, it was observed that these substances strongly inhibited the 11 β -hydroxylation of either 11-deoxycorticosterone or 17-hydroxycorticosterone (compound *S*). Further study indicated that a large series of inorganic ions (sodium sulphate, sodium chloride, potassium chloride, calcium chloride, dipotassium hydrogen phosphate, magnesium chloride, lithium sulphate) was capable of inhibiting the hydroxylation reaction.

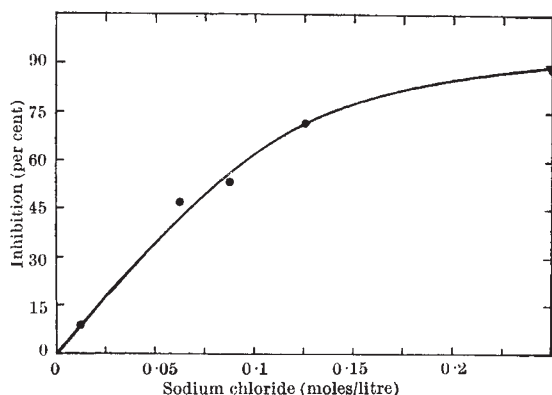


Fig. 1. The reaction mixture contained reduced triphosphopyridine nucleotide, 0.2 μ mole; deoxycorticosterone, 0.058 μ mole; tris(hydroxymethyl)aminomethane buffer, pH 7.4, 8 μ mole; enzyme; and added salt as indicated, in a volume of 1.0 ml. 10 min. incubation at 37° C. in air.

In Fig. 1, which illustrates this phenomenon when sodium chloride was the inhibitor, it can be seen that inhibition of corticosterone formation from 11-deoxycorticosterone was proportional to the concentration of added salt. A similar inhibitory action of ions has been observed previously with glutamic dehydrogenase⁴, and other enzymes.

Although current physiological evidence⁵ suggests that intracellular ionic strength is not a primary factor in the regulation of adrenal steroid output, the observation presented here seems of particular interest since the adrenal cortex, by virtue of its ability to synthesize 11-hydroxylated salt-retaining hormones, is, in part, responsible for the maintenance of a constant ionic environment *in vivo*.

GORDON M. TOMKINS
PATRICIA J. MICHAEL

National Institute of Arthritis
and Metabolic Diseases,
National Institutes of Health,
Bethesda, Maryland.
April 12.

¹ Hayano, M., and Dorfman, R. I., *J. Biol. Chem.*, **211**, 227 (1954).

² Brownie, A. C., and Grant, J. K., *Biochem. J.*, **62**, 29 (1955).

³ Tomkins, G. M., Michael, P. J., and Curran, J. F., *Biochim. Biophys. Acta*, **23**, 655 (1957).

⁴ Olsen, J. A., and Anfinsen, C. B., *J. Biol. Chem.*, **202**, 841 (1953).

⁵ Bartter, F. C., Liddle, G. W., Duncan, jun., L. E., Barber, J. K., and Delea, C., *J. Clin. Invest.*, **35**, 1306 (1956).

Composition of the Cell Wall of *Lactobacillus bifidus*

ANALYSES of hydrolysates of cell walls from a large number of different Gram-positive bacteria led to the suggestion that the chemical composition of bacterial cell walls may be a valuable taxonomic character. In general, the particular amino-acids present in the cell wall appeared to be characteristic of the genus, while specific and to a lesser extent strain differences were reflected in the type and relative amounts of the sugars and hexosamines present¹.

We have recently examined the cell-wall compositions of eight strains of *Lactobacillus bifidus*. The results are of interest both because they may throw some light on the taxonomic position of *L. bifidus*, and because they reveal an unusual degree of variation among different strains of what is thought to be a single bacterial species.

The strains investigated were some of those which had previously been examined by Hayward, Hale and Bisset², and we are indebted to these workers for making them available to us. For examination, the organisms were grown in tomato-juice broth³ at 37° C., in an atmosphere of hydrogen plus 10 per cent carbon dioxide. After 2-4 days growth the cultures were killed by the addition of formalin, and the washed suspensions disintegrated in a Mickle shaker. Purified cell walls were prepared by digesting the insoluble fraction with trypsin and pepsin¹, and samples were hydrolysed at 100° C. in 2 *N* sulphuric acid for 2 hr. (sugars) or 6 *N* hydrochloric acid for 8 hr. (amino-acids).

The results of analysis by paper chromatography of hydrolysates of the cell walls of these strains are shown in Tables 1 and 2. In recording the amino-acids (Table 1) trace amounts have been ignored to avoid undue complication of the table. Alanine, glutamic acid and lysine were present in fractions from all strains, as were glucosamine and the amino-