control and carbenoxolone-treated adrenalectomized rats were fractionated by the method of Herman *et al.* (1968) into nuclear residue, a 50%-(w/v)-(NH₄)₂SO₄ precipitate and a 50%-(w/v)-(NH₄)₂SO₄ supernatant. After administration of [³H]aldosterone (0.15 nmol) the concentrations (fmol/mg of protein) of [³H]aldosterone in these three fractions were 1.5, 16.0 and 9.6 respectively in control rats and 1.4, 12.0 (*P* < 0.05) and 7.2 (*P* < 0.05) respectively in carbenoxolone-treated rats. Herman *et al.* (1968) have demonstrated that the (NH₄)₂SO₄-precipitate fraction contains stereospecific mineralocorticoid-binding macromolecules. Our experiments with rat kidney as a model system indicate that carbenoxolone can inhibit the binding of aldosterone in the kidney. Therefore alterations in the distribution of aldosterone may be the basis of the mineralocorticoid side effects exhibited by some patients treated with the drug.

Financial support for M. J. H. from Biorex Laboratories Ltd. is gratefully acknowledged.

Baron, H. J. & Sullivan, F. M. (eds.) (1970) *Carbenoxolone Sodium (Original Papers and Discussion of a Group Meeting)*, Butterworths, London

Baron, J. H., Nabarro, J. D. N., Slater, J. D. H. & Tuffley, R. (1969) *Brit. Med. J.* ii, 793-795

Doll, R., Langman, M. J. S. & Shawdon, H. H. (1968) in *A Symposium on Carbenoxolone Sodium* (Robson, J. M. & Sullivan, F. M., eds.), pp. 51-57, Butterworths, London

Fanestil, D. D. & Edelman, I. S. (1966) *Proc. Nat. Acad. Sci. U.S.A.* 56, 872-879

Hausmann, W. & Tarnoky, A. L. (1968) in *A Symposium on Carbenoxolone Sodium* (Robson, J. M. & Sullivan, F. M., eds.), pp. 159-172, Butterworths, London

Herman, T. S., Fimognari, G. M. & Edelman, I. S. (1968) *J. Biol. Chem.* 243, 3849-3856

Porter, G. A. (1970) in *Carbenoxolone Sodium (Original Papers and Discussion of a Group Meeting)* (Baron, J. H. & Sullivan, F. M., eds.), pp. 33-47, Butterworths, London

Hepatic and Extrahepatic Reduction of Nicotine 1'-N-Oxide in Rats

By R. M. DAJANI, J. W. GORROD and A. H. BECKETT
[Department of Pharmacy, Chelsea College (University of London), London SW3 6LX, U.K.]

Reduction of nicotine 1'-N-oxide [7:3 mixture of *trans* and *cis* diastereoisomers from *S*-(-)-nicotine] to nicotine has already been demonstrated in humans after oral administration (Beckett *et al.*, 1970) and by guinea-pig liver preparations (Booth & Boyland, 1971). We now report the hepatic and extrahepatic reduction of nicotine 1'-N-oxide by rats, and present evidence for the localization and characterization of

the enzymes involved. Enzyme activity was measured by determining the nicotine produced. The analytical procedures used were as described by Beckett *et al.* (1971).

Nicotine 1'-N-oxide was reduced anaerobically by all tissues studied, the liver being the most active. The small intestine was the most active, on a wet-weight basis, of the extrahepatic tissues, having 40-65% of the activity found in the liver, whereas kidney, heart, lung and spleen had only 5-10% of the hepatic activity. Blood or brain had no significant reducing ability towards nicotine 1'-N-oxide under the conditions used. Reduction was also observed on anaerobic incubation of nicotine 1'-N-oxide with suspensions of rat intestinal contents.

Almost all the activity from either liver or intestine was present in the 1000g supernatant, and the bulk of this was not sedimented at 10000g. The activity was present in both the microsomal and the soluble fractions, the former having 2-3 times the activity of the latter. The microsomal and soluble-fraction reductases were non-specific with regard to their requirements for NADH or NADPH, although the latter produced a greater degree of reduction.

Both reducing systems of the soluble and microsomal fractions were partially inhibited by either SKF 525-A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) or EDTA (1 mM) and nearly completely inhibited by KCN (1 mM) or CO with either NADH or NADPH as cofactor. Practically all reducing activity was abolished by boiling the tissue or by incubation in the presence of air. NADH or NADPH in the absence of tissue failed to reduce nicotine 1'-N-oxide.

It appears that the reducing systems involve an NADPH or NADH cytochrome reductase, and possibly cytochrome P-450. The behaviour of the systems towards inhibitors, heat and O₂ suggests a similarity with nitro reductase.

R. M. D. is presently on sabbatical leave from The American University, Beirut, Lebanon.

Beckett, A. H., Gorrod, J. W. & Jenner, P. (1970) *J. Pharmacol.* 22, 722-723

Beckett, A. H., Gorrod, J. W. & Jenner, P. (1971) *J. Pharm. Pharmacol.* 23, 55S-61S

Booth, J. & Boyland, E. (1971) *Biochem. Pharmacol.* 20, 407-415