The mode of action of the hormone in raising sensitivity to these centrally acting drugs is not clear, but the results point to possible difficulties in drug therapy in hyperthyroid patients.

† Present address: Biological Research Department, B.D.H. (Research) Ltd., Borough Road Godalming, Surrey.

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Diuretics and carbohydrate metabolism in the mouse

J. M. FOY and B. L. FURMAN* (introduced by G. D. H. LEACH), *Postgraduate School of Studies in Pharmacology, University of Bradford*

Numerous reports appear in the literature concerning the so-called diabetogenic effect of benzothiadiazine diuretics (for example, Goldner, Zarowitz & Akgun, 1960). Recent reports suggest that frusemide (Toivonen & Mustala, 1966) and ethacrynic acid (Lebacq & Marcq, 1967) may also produce impairment of glucose tolerance in some patients. It is not yet clear whether or not these compounds can produce these abnormalities in subjects exhibiting no predisposition towards diabetes mellitus. Attempts to induce abnormal carbohydrate metabolism in normal animals with benzothiadiazine compounds have yielded conflicting results.

In this communication results are presented to show the effects of short-term (14 days) treatment with hydrochlorothiazide, ethacrynic acid and frusemide on fasting blood glucose levels, oral glucose tolerance, fasting plasma levels of immuno-reactive insulin, and insulin sensitivity.

Fasting blood glucose determinations were carried out on 0·05 ml. blood samples using a micro-colorimetric copper reduction method. The results indicate a small but significant fasting hyperglycaemia compared with controls, in animals treated with large daily doses (100 mg/kg) of frusemide or ethacrynic acid but not with hydrochlorothiazide. Low doses of the diuretics produced no effect.

Fasting plasma levels of immuno-reactive insulin were determined by the method of Hales & Randle (1963). Elevation in the levels was seen in animals treated with ethacrynic acid and frusemide, statistical significance being shown in the latter case.

Oral glucose tolerance was assessed by measuring blood glucose in groups of mice sampled 30, 60, 90, 120 and 180 min after an oral glucose load (5 g/kg). Significant elevations of blood glucose compared with control animals were seen at 30 and 60 min in the ethacrynic acid treated animals and at 60 and 90 min in the frusemide-treated animals. No change was observed with hydrochlorothiazide or with low doses of the other diuretics.

The insulin sensitivity of the animals was assessed by measuring blood glucose before and 30 min after insulin (0·5 u./kg), administered intravenously. No diuretic-induced change was observed.

The latter observation, together with the observation that increases in plasma insulin do not appear disproportionate in relation to the elevated fasting blood glucose level, suggest that there is no insulin-resistance in treated animals. It is therefore possible that altered glucose tolerance is due to a delayed response of the pancreatic β cells to acute increases in blood glucose, or to an alteration in the rate of gastro-intestinal absorption of glucose. These possibilities are under investigation.

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A possible relationship between depletion of noradrenaline and blockade of adrenergic neurones.

E. T. ABBS* and M. I. ROBERTSON, *Department of Pharmacology, School of Pharmacy, College of Technology, Portsmouth*

Several hypotheses have been advanced to explain the mode of action of adrenergic neurone-blocking agents. One hypothesis, that adrenergic neurone blockade might be associated with depletion of catecholamines, was tested by Cass & Spriggs (1961). They found no correlation between tissue catecholamine levels and the blockade produced by bretylium or guanethidine.

Abbs (1966) suggested that there might be a selective depletion from a small "compartment" of the "noradrenaline store" which might be associated with adrenergic neurone blockade. The present studies, employing subcellular fractionation procedures, were undertaken to test this hypothesis. Cats were anaesthetized with ether and chloralose (80 mg/kg intravenously) and their spleens were removed and homogenized in 0·25 M sucrose containing 0·001 M $MgCl_2$ and 0·005 M phosphate buffer, pH 7·4. Subcellular fractions were prepared by differential centrifugation of an aliquot of the homogenate. A coarse pellet (P_1 fraction) was produced by centrifuging at 12,000 *g* for 10 min and the resulting supernatant fluid was then centrifuged at 100,000 *g* for 1hr, yielding a small pellet (P_2 fraction) and a supernatant layer (S fraction). Noradrenaline was extracted from the fractions and also from an aliquot of uncentrifuged homogenate (T). The extracts were then purified and the noradrenaline was assayed fluorimetrically as described by Abbs (1966).

Treatment of the cats for various times with bretylium tosylate (10 mg/kg intravenously) produced depletion of noradrenaline in subcellular fractions at times when adrenergic neurone blockade was evident but when it was not possible to demonstrate such a depletion in the unfractionated homogenate. Both the depletion of noradrenaline and the development of the adrenergic neurone blockade were prevented by previous administration of (+)-amphetamine sulphate (2·5 mg/kg intravenously). The significance of this selective depletion of noradrenaline by bretylium will be discussed in relation to adrenergic neurone blockade.

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