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.

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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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Self-stimulation of the brain after administration of an amphetamine-barbiturate mixture

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In 1961 Steinberg, Rushton & Tinson discovered that certain small doses of amphetamine sulphate and amylobarbitone sodium administered as a mixture produced striking increases in the locomotor activity of rats in an unfamiliar Y-maze. Other doses (in a mixture of amphetamine–amylobarbitone of constant ratio 1 : 20) were much less effective (Rushton & Steinberg, 1963). Each of the constituents produced small increases in locomotor activity, but the effect of the mixture could not be explained on an additive model. Dose-response curves, very similar to those of Rushton & Steinberg (1963), have been obtained with mice, in a different kind of exploratory situation (Bradley, Joyce, Murphy, Nash, Porsolt, Summerfield & Twyman, 1968).

The effect of the mixture on learning to press a bar to obtain water has been investigated in water-restricted rats (Joyce & Summerfield, 1966). It is known (Epstein, 1959; Schmidt & Dry, 1963) that the constituent drugs of the mixture can affect spontaneous drinking behaviour and that these effects of the two drugs are in opposite directions. The possibility that amylobarbitone and amphetamine affect motivational factors involved in learning or performing the task thus complicates the interpretation of results obtained with the mixture. Olds & Milner (1954) have demonstrated that satiated rats will learn to press a bar to deliver a brief train of electrical stimulation to a subcortical region of the brain. This " rewarding " effect of electrical stimulation applied directly to the brain maintains bar-pressing. The effect of an amphetamine–barbiturate mixture on this behaviour was analysed for individual animals.

Male rats learned to press a bar to deliver an electrical stimulus to the brain (60 Hz sine wave, 0·5 sec duration, 40–125 μA) in the region of the lateral hypothalamus. The effects of giving intraperitoneally a mixture of amphetamine sulphate (0·75 mg/kg) and amylobarbitone sodium (15 mg/kg) were then compared with those of giving the separate constituents or saline. At the three current intensities tested the mixture, like amphetamine, increased response rate in each animal; amylobarbitone usually slightly decreased response rate but occasionally increased it. There was no evidence that amylobarbitone and amphetamine given together acted antagonistically. At the highest current intensity (40 μA above threshold for each rat) all animals tested after administration of the mixture pressed the bar at a higher rate than after amphetamine ($P < 0{\cdot}01$) regardless of whether after amylobarbitone their response rate increased or decreased.

These results provide further evidence that the " special " enhancement effect of the mixture (Rushton & Steinberg, 1963) cannot be explained on an additive model