position. There is no question that cholesterol synthesis occurred, since acetate was readily incorporated into the product. On the other hand, digoxin and digitoxin were not incorporated into cholesterol.

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Effects of *d*-Amphetamine and Chlorpromazine on Oxidised (NAD) and Reduced ($NADH_2$) Nicotinamide Adenine Dinucleotide Levels in Rat Brain

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LARGE doses of nicotinamide increase liver, brain and spleen NAD.[1, 2] Nicotinic acid has a similar effect on blood NAD.[3, 4] Reserpine or chlorpromazine, given prior to nicotinamide, maintain elevated liver NAD levels.[5] The ability of brain tissue to control NAD metabolism is reflected by the absence of this reserpine effect in brain and because nicotinamide itself causes only a 50–75% increase in brain NAD[2] but an 800–900% increase in the liver.[1] Furthermore, when peripheral NAD stores are severely depleted by dietary deficiences,[6] no change in brain NAD occurs, even in terminally deficient animals.[2]

We have investigated the effects of *d*-amphetamine sulphate and chlorpromazine hydrochloride on rat brain NAD and $NADH_2$ levels to determine if the influence of these drugs on behaviour could be correlated with changes in NAD metabolism.

Drug solutions were prepared in 0·9% NaCl so that the required dose was contained in 0·2 ml/100 g body weight. Groups of two or three male Wistar rats of approximately equal weight (90–120 g) were used. The order in which the animals were injected intraperitoneally, killed and the brains dissected, extracted and assayed, was randomised, using either a 3 × 3 Latin Square design or a table of random numbers. 0·9% NaCl was used as control. The method for extracting and estimating the nicotinamide

nucleotides was essentially that of Lowry *et al.*,[7] using the whole brain instead of a sample. The experimental data were evaluated by means of the appropriate variance analysis.

The results are shown in Table 1. Only *d*-amphetamine (10 mg/kg) produced a statistically significant fall in the NAD content. No significant changes were observed in the $NADH_2$ levels.

TABLE 1. *In vivo* EFFECTS OF *d*-AMPHETAMINE AND CHLORPROMAZINE ON RAT BRAIN NAD AND $NADH_2$ LEVELS

Series	Treatment	Dose (mg/kg)	Time after treatment (hr)	Concentration (μmoles/g) NAD	$NADH_2$
1	Control		3	0·198 ± 0·011	0·020 ± 0·003
	d-Amphetamine	5		0·170 ± 0·012	0·021 ± 0·004
	d-Amphetamine	2·5		0·164 ± 0·010	0·020 ± 0·003
2	Control		3	0·184 ± 0·010	0·023 ± 0·005
	Chlorpromazine	30		0·201 ± 0·006	0·023 ± 0·003
	Chlorpromazine	15		0·156 ± 0·009	0·021 ± 0·003
3	Control		2	0·191 ± 0·013	0·024 ± 0·003
	d-Amphetamine	2·5		0·176 ± 0·010	0·025 ± 0·004
4	Control		2	0·192 ± 0·005	0·029 ± 0·004
	d-Amphetamine	5		0·187 ± 0·006	0·030 ± 0·003
5	Control		2	0·198 ± 0·005	0·030 ± 0·004
	d-Amphetamine	10		0·163 ± 0·003*	0·028 ± 0·004

All values are the means (± S.E.M.) of 9 or 10 determinations. Significance of difference from control: *(0·01 > P > 0·001).

The suggestion, based on investigations on the liver,[5] of an association between NAD metabolism and the pharmacological activities of reserpine, has not been confirmed for brain.[2] Nevertheless, since massive doses of nicotinamide, sufficient to produce marked lethargy in experimental animals,[8] have been used to treat schizophrenia,[9] it is possible that the modification of NAD metabolism may be a factor in the action of psychotropic drugs. Supporting evidence has been provided by studies revealing a correlation between the behavioural effects of certain psychotropic drugs and their abilities to alter brain levels of adenine nucleotides,[10-13] which are involved in the metabolism of nicotinamide nucleotides.[14]

The results obtained with *d*-amphetamine (10 mg/kg) are compatible with those of Lewis and Van Petten,[10, 12] who showed that a number of behavioural stimulants, including *d*-amphetamine and iproniazid, increase brain ATP levels. However, lack of an effect in these preliminary experiments with chlorpromazine, which produces a fall in brain ATP,[15] prevents a comparable inverse relationship between brain ATP and NAD levels being postulated for this drug.

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The inhibition of some glycolytic enzymes by chlorambucil*

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Chlorambucil {*p*[bis(2-chloroethyl)amino]phenylbutyric acid} has recently been the subject of investigations in antitumor studies.[1–3] Since chlorambucil increases blood glucose levels in rat,

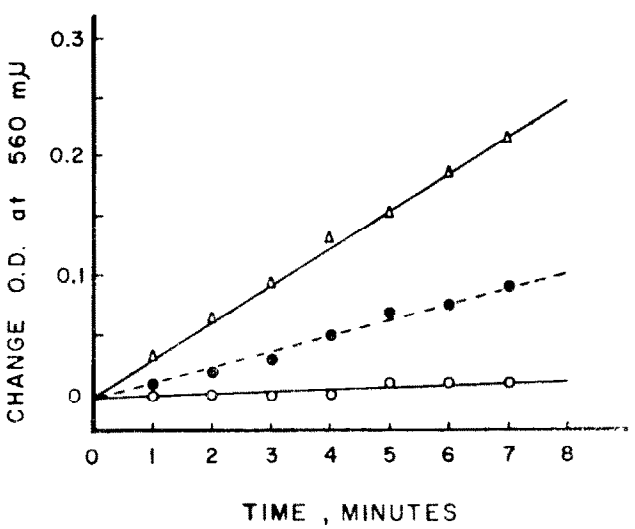

Fig. 1. The effect of chlorambucil on hexokinase. The final assay mixtures contained 4·47μg hexokinase; △, control hexokinase; ●, hexokinase preincubated with 2×10^{-3} M chlorambucil; ○, hexokinase preincubated with 4×10^{-3} M chlorambucil

inhibits respiration of yeast more significantly with glucose as an energy source than does citrate or succinate,[4] and lowers the glycogen content of lymphocytes,[5] its effect on several glycolytic enzymes was investigated in order to correlate the observed action on whole cells and organisms.

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