TABLE 2. ALA DEHYDRASE OF NORMAL AND PORPHYRIC RAT TISSUES

	Activity* (μmoles PBG/hr per g wet wt)											
Normal	1	2	3	4	5	6	7	8				Av.
Liver	0·70	0·77	0·63	0·71	0·71	0·70	0·70	0·75				0·72
Kidney	0·22	0·26	0·23	0·23	0·28	0·23						0·24
Porphyric (all Sedormid)	9	10	11	12	13	14	15	16	17	18	19	Av.
Liver	1·37	1·00	1·41	1·09	1·47	1·22	1·18	1·31	1·11	1·41	1·42	1·27
Kidney	0·56	0·50	0·62	0·43	0·54	0·43						0·51

* Each value is the average of three determinations in each rat. Activity in porphyric liver: 76% over the normal. Activity in porphyric kidney: 112% over the normal.

patients. The urinary excretion of ALA and PBG observed in our animals may be a reflection of the levels of these precursors in liver and kidney. The present data would then agree and explain our previous results[1], which showed a greater urinary excretion of ALA, a slight increase of PBG, uroporphyrins, and coproporphyrins in rats, and a minor increase in the level of ALA excretion together with much greater excretions of PBG, uroporphyrins and coproporphyrins, in rabbits. The observed urinary excretion levels would then reflect the dehydrase activity in liver and in kidney.

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Effects of hydrazine and alkylhydrazines on carbohydrate metabolism of rat brain

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IN A previous study[1] it was observed that hydrazine (HY), unsymmetrical dimethylhydrazine (UDMH), symmetrical dimethylhydrazine (SDMH), and monomethylhydrazine (MMH) produced an initial hyperglycemia after injection into rats. The present report describes observations on the metabolism of carbohydrates in brains of poisoned rats.

EXPERIMENTAL

Rats were decapitated 75 min after i.p. injection of HY, UDMH, and SDMH, and 35 min after MMH; these times were selected to precede convulsions.[1] The doses used were the LD_{50} for HY, UDMH, and MMH (64, 102, and 28 mg/kg, expressed as free base respectively) and 500 mg/kg for SDMH, which is not toxic to rats. The brains were removed as quickly as possible (1–2 min); the cerebral cortex, cerebellum, and medulla oblongata were removed.

Anaerobic and aerobic respiration

These studies were carried out with slices prepared with a McIlwain tissue chopper. The CO_2 output was measured anaerobically in Krebs-Ringer bicarbonate buffer[2] on cortex only, in three rats per treatment, with five samples from each. The O_2 uptake was measured with Tris buffer[3] and: (a) with 0·1 M glucose as substrate, in the three brain areas, with three rats per treatment on triplicate sample of cortex and duplicate samples of other areas; (b) with 0·1 M lactate or succinate, on cortex only, with two rats per treatment and triplicate samples; (c) without substrate, on cortex only, two rats per treatment and duplicate samples.

Lactates

The tissues were rapidly removed, weighed, transferred to tubes containing 2 ml 10% trichloroacetic acid, and homogenized with a Potter–Elvehjem homogenizer; then 2 ml 0·9% NaCl was added, and the mixture was centrifuged. Lactate concentrations were determined according to Barker and Summerson.[4] It was not possible to use rapid-freezing techniques because these made it impossible to dissect the brain into the required parts.

RESULTS

The treatments with hydrazine or alkylhydrazines had no significant effects upon the O_2 uptake or CO_2 output, except that the O_2 uptake of medulla slices from SDMH-treated rats was 28 per cent greater than controls. The 't' test at the 0·05 level was used to determine significance. The addition of lactate caused an inhibition of O_2 uptake, as previously observed,[5, 6] in slices from brains of normal and poisoned rats.

HY, SDMH, and UDMH all caused a dramatic lowering in the lactate levels of all three areas of brain, the most extreme case being a 70% reduction by UDMH (Table 1). By contrast, MMH had very little effect.

TABLE 1. ENDOGENOUS LACTATES OF FRESH RAT BRAIN*

Tissue	Control	HY	SDMH	MMH	UDMH
Cortex	115·4 ± 4·93	55·4 ± 4·4	70·13 ± 3·12	114·6 ± 5·14	34·44 ± 1·20
Cerebellum	115·2 ± 4·60	68·5 ± 4·39	73·91 ± 6·61	145·6 ± 8·27	52·4 ± 3·22
Medulla oblongata	117·6 ± 5·25	73·6 ± 7·44	86·49 ± 4·20	129·8 ± 7·28	47·9 ± 4·31

* Values are in micromoles per gram (mean ± S. E.). Each cortex figure represents the mean of nine samples from three individuals. Cerebellum and medulla oblongata figures represent six samples from three individuals. All assays were run in duplicate. For HY, SDMH, and UDMH, all values are significantly less than control, for 't' = 0·01. For MMH, the cortex and medulla values are not significantly different from controls even at 't' = 0·05; the cerebellum value is significantly higher at 't' = 0·05 but not at 't' = 0·01.

The experiments with slices shed no light on the large effects upon endogenous lactate. Since SDMH is not toxic to rats under our conditions and causes large lactate reductions, and since MMH is highly toxic and causes no lactate reduction, it is unlikely that the effects upon lactate are causally related to toxicity.

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Effects of stress and D-amphetamine on rat brain catecholamines*

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CERTAIN stressful environmental factors are known to influence the excitatory and lethal actions of D-amphetamine,[1, 2] and some of these influences may be exerted through the release of norepinephrine from endogenous stores.[3] For example, in individually caged mice, D-amphetamine causes partial depletion of brain and heart norepinephrine stores; aggregation or crowding of mice markedly enhances the toxicity and norepinephrine-depleting actions of this drug. We,[4] as well as others,[5] have reported that in 'nonstressed' rats D-amphetamine causes a reduction in the norepinephrine content of brain; it does not affect the brain content of dopamine. The present study was initiated in an effort to determine what effects various 'stresses' have on the ability of D-amphetamine to deplete the catecholamines in rat brain.

Sprague–Dawley female rats weighing 180–220 g were randomly divided into seven groups of twelve each (one control and six stressed groups). Each group was divided in half; six rats received an i.p. injection of D-amphetamine (3 mg/kg) in a volume of 5 ml isotonic saline/kg, and the other six received equal volumes of saline. The rats were then subjected to various stressful situations. After a period of 4 hr (1 hr for grid shock series) the animals were sacrificed and the norepinephrine and dopamine content of the brains determined as previously described.[4]

The stressful procedures used in this study were as follows.

Restraint. Rats were placed singly in adjustable wire-mesh cages that prevented changes in posture for a 4-hr period.

Swim. The animals were forced to swim in individual containers for 4 hr. The temperature of the water was maintained at 23° for one series (12 animals) and at 37° for another series (12 animals).

Tail shock. Four rats were placed in restraint cages and were wired in series with alligator clips attached to their tails. The tails were shocked every 5 sec with 175 V square-wave pulse of 1 sec duration (laboratory stimulator AEL 104A). Polarity was switched every 2 min. Under these conditions the shocks caused the rats to squeal and twitch their tails.

Sound. Rats were placed in individual cages in a small enclosed room and subjected to a loud tone (4,000 c/s) for 2 sec every 5 sec. The animals reacted to the tone for the first few minutes, but within 30 min adapted to it so that it no longer appeared to distress them.

Grid shock. Two rats (one treated with saline and one with D-amphetamine) were placed in separate compartments of a Skinner box containing a floor of stainless steel rods through which an electric shock could be delivered. Every 10 sec each rat was subjected to a shock of 1·6 mA intensity and 1 sec duration. Preliminary studies showed that the D-amphetamine-treated animals began to die if kept in the shocking cages for longer than 1 hr.

After exposure to the sound, restraint, and tail shock procedures, the general appearance of the saline- and D-amphetamine-treated rats was not different from the nonstressed rats receiving saline and D-amphetamine. There were no deaths in these series. In the swim series both the saline- and D-amphetamine-treated rats were depressed and exhausted at the time of sacrifice. Several animals