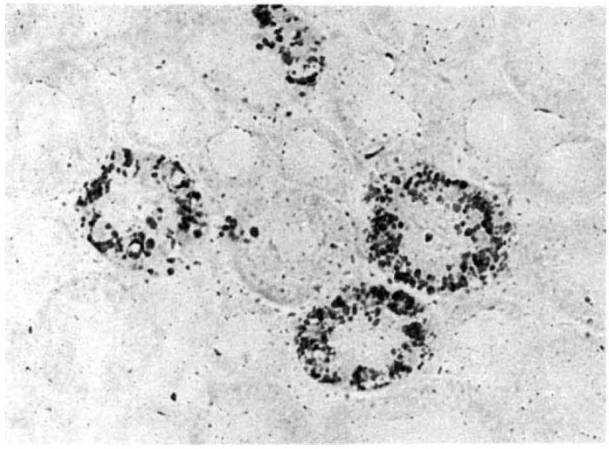

Fig. 2. Lipid aggregates in the renal tubules.

We found, however, that if frozen plasma was thawed more rapidly by standing in warm water at 56°–60° C, a cloudy flocculation formed in the plasma, which was removed by filtration ('Millipore' 0·22μ). When this cleared plasma was used as the perfusate, there was no rise in perfusion pressure, no oedema of the kidney and no cell damage, even after many hours of perfusion at high flow rates (1 ml./min/g of kidney tissue).

Investigations showed that 30–35 per cent of the total plasma lipids was removed from the plasma by freezing, rapid thawing and filtration. Chromatography on the filter residues indicated that they were mostly lipid materials. Possibly these were derived from unstable lipid or lipoprotein substances which were little affected by slow thawing of plasma at room temperature, but became denatured during perfusion at hypothermic temperatures, liberating the lipid complexes which damaged the kidney. The detailed nature of the substances removed from the plasma by freezing and rapid thawing is not yet fully elucidated, but further investigations are in progress.

Successful preservation of kidneys from canine and human corpses has been possible using hypothermic perfusion with homologous plasma which has been treated in this way. We have had no further trouble with rising perfusion pressure, and histological studies on frozen section biopsies have shown complete absence of lipid material from the kidneys. The high flow rates that were maintained for long periods allowed adequate oxygenation with the oxygen in simple solution in the plasma, only using air in the oxygenator.

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[1] Belzer, F. O., Ashby, B. S., and Dunphy, J. E., *Lancet*, ii, 536 (1967).

Block by LSD of the Increase in Brain Serotonin Turnover induced by Elevated Ambient Temperature

An increased turnover of brain serotonin (5-hydroxytryptamine; 5-HT) occurs in rats exposed to high environmental temperature; when the synthesis of 5-HT is blocked a more rapid depletion of brain 5-HT occurs at an ambient temperature of 40° C (ref. 1). In addition, the concentration of the principal metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), is increased in the brain at this temperature (personal communication from R. K. McDonald). The mechanism of this activation of 5-HT turnover at high environmental temperature is not known, but a similar increase in 5-HIAA is seen when the neurones containing 5-HT (situated in the caudal midbrain raphé[2]) are electrically stimulated[3,4]. It follows that the increased turnover of 5-HT at high ambient temperatures could result from an acceleration of the firing rate of the neurones containing 5-HT. (+)-Lysergic acid diethylamide (LSD) completely inhibits the firing of neurones containing 5-HT (ref. 5). We have given LSD to rats exposed to high environmental temperatures to test the possibility that the increase in brain 5-HIAA found in these conditions is prevented by a drug which inhibits the firing of 5-HT-containing neurones.

Sprague–Dawley (Charles River) male rats weighing 150–250 g were used. Before the experiment the rats were placed in individual cages and kept in a quiet room at 23° C for 4 h. An adaptation period is important for establishing uniformly low baseline levels of 5-HIAA because any alteration in environment, including transfer to a new cage, can induce a temporary increase in brain 5-HIAA concentration (unpublished results of A. Schwartz and G. K. A.). After the adaptation period, the rats (in individual cages) were either placed in an incubator at 40° C for 45 min or allowed to remain at 23° C for 45 min. Some rats received intraperitoneal injections of LSD (bitartrate salt), the bitartrate salt of 2-brom-lysergic acid diethylamide (BOL), or an equivalent volume of saline free of pyrogen just before the 45 min test period. Immediately after the 45 min had elapsed, the rats were decapitated (no anaesthesia) and their brains were removed and frozen before fluorimetric assay for 5-HT and 5-HIAA. The assay procedure we used[4] incorporated modifications of the methods of Bogdanski *et al.*[6], Udenfriend *et al.*[7] and Wise[8].

Table 1. EFFECT OF LSD AND BOL ON BRAIN 5-HIAA AND 5-HT CONCENTRATIONS IN THE RAT AFTER 45 MIN AT AMBIENT TEMPERATURES OF 23° C OR 40° C

No. of animals	Drug	Ambient temperature (° C)	5-HIAA ng/g (+S.E.M.)	Per cent change	P*	5-HT ng/g (+S.E.M.)	Per cent change	P*
8	No injection	23	321 ± 7	—	—	430 ± 12	—	—
4	No injection	40	471 ± 23	+46·7	< 0·001	408 ± 18	−5·1	N.S.
12	Saline	40	439 ± 11	+36·8	< 0·001	397 ± 13	−7·7	N.S.
4	LSD 500 μg/kg	23	293 ± 13	−8·7	< 0·05	444 ± 3	+3·2	N.S.
4	LSD 500 μg/kg	40	313 ± 14	−2·5	N.S.	425 ± 16	−1·2	N.S.
4	LSD 250 μg/kg	23	292 ± 3	−9·0	< 0·02	410 ± 13	−4·6	N.S.
4	LSD 250 μg/kg	40	320 ± 12	−0·3	N.S.	416 ± 18	−3·2	N.S.
4	BOL 300 μg/kg	40	442 ± 16	+37·7	< 0·001	408 ± 18	−5·1	N.S.

* *P* values were determined using Student's *t* test. *S.E.M.*, standard error of the mean.

There was an increase of approximately 40 per cent in the concentration of 5-HIAA in the brains of animals kept at 40° C for 45 min (Table 1). We thus confirm the finding of McDonald that an elevated ambient temperature induces an increase in brain 5-HIAA concentration. It can be seen from Table 1 that this increase is blocked by previous injection of 250 or 500 μg of LSD. In contrast, BOL, the non-psychoactive congener of LSD, is ineffective in preventing the increase in 5-HIAA concentration. In other experiments it was found that if the period of incubation at 40° C was longer than 45 min, previous injection of LSD (250 μg/kg) did not block increases in 5-HIAA entirely. Diminution of the effect of LSD after 45 min can be correlated with its loss through metabolic destruction[9]. In accord with the findings of Rosecrans *et al.*[9], LSD produced a significant decrease (9 per cent) in 5-HIAA in rats kept at 23° C compared with control rats at the same temperature. At the elevated temperature this depression after LSD is

abolished. None of the differences in 5-HT concentrations is significant ($P > 0.05$) when compared with the controls at room temperature or with each other.

Colonic temperatures were recorded for a group of four rats given 250 μg/kg of LSD and four rats receiving an equivalent volume of the pyrogen-free saline. The rats were first allowed to adapt in a quiet room for 4 h at 23° C, then were injected and placed in an incubator at 40° C for 45 min. The mean colonic temperature for the LSD group before incubation was 37·47° C (*S.E.M.* ± 0·11) and for the saline group, 37·57° C (*S.E.M.* ± 0·21). The values after incubation were 41·80° C (*S.E.M.* ± 0·43) for the LSD rats, and 40·75° C (*S.E.M.* ± 0·2) for the saline rats. Although LSD prevented the rise in 5-HIAA concentration, it did not prevent a rise in body temperature.

These results demonstrate that LSD (but not its non-psychoactive congener, BOL) blocks the accelerated turnover of 5-HT induced by the exposure of rats to an elevated ambient temperature. This is consistent with the hypothesis that LSD prevents an increase in 5-HIAA concentration by blocking an activation of firing of the neurones containing 5-HT. LSD could, however, have an inhibitory influence on 5-HT metabolism by means other than that of an effect on neuronal firing. For instance, LSD might prevent increases in 5-HIAA by directly enhancing the "binding" or storage of 5-HT, by inhibiting its release[10] or by inhibiting monoamine oxidase. But LSD interferes with the binding of 5-HT *in vitro*[11] and has no inhibitory effect on monoamine oxidase[12]. The fact that LSD does inhibit the firing of neurones containing 5-HT represents a sufficient, if not exclusive, explanation for the block observed in 5-HT metabolism. Inhibition of the firing of 5-HT-containing neurones by LSD may, however, be an indirect rather than a direct effect.

Although there have been many studies concerned with the possible role of 5-HT in the regulation of body temperature (for example, experiments involving intraventricular injections of 5-HT (refs. 13–15), pharmacological manipulation of 5-HT concentration in the brain[16,17], measures of amine metabolism after induction of fever with leucocytic pyrogen[18], and the direct stimulation of neurones containing 5-HT (ref. 19)), no definitive conclusion can be drawn about the role (if any) of brain 5-HT in temperature regulation. Our study was not directly concerned with temperature regulation. We have merely utilized, as a model system, the activation of 5-HT turnover by high ambient temperatures to test the possibility that LSD, a drug which inhibits the firing of neurones containing 5-HT, may similarly block an activation of metabolic turnover. It is therefore significant that the observed inhibitory effect of LSD on 5-HT metabolism exactly parallels data[5] on the time course of the LSD effect on the firing of 5-HT-containing neuronal units. To our knowledge this is the first instance in which an effect of a drug on the metabolism of a presumptive transmitter substance in the brain has been predicted on the basis of known effects of the drug on the unit activity of the specific neurones containing that substance.

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[1] Corrodi, H., Fuxe, H., and Hökfelt, T., *Acta Physiol. Scand.*, **71**, 224 (1967).
[2] Dahlström, A., and Fuxe, K., *Acta Physiol. Scand.*, **62**, Suppl., 232, 1 (1965).
[3] Aghajanian, G. K., Rosecrans, J. A., and Sheard, M. H., *Science*, **156**, 402 (1967).
[4] Sheard, M. H., and Aghajanian, G. K., *J. Pharmacol. Exp. Therap.* (in the press).
[5] Aghajanian, G. K., Foote, W. E., and Sheard, M. H., *Science*, **161**, 706 (1968).
[6] Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S., *J. Pharmacol. Exp. Therap.*, **117**, 82 (1956).
[7] Udenfriend, S., Weissbach, H., and Brodie, B. B., *Meth. Biochem. Anal.*, **6**, 95 (1958).
[8] Wise, C. D., *Anal. Biochem.*, **20**, 369 (1967).
[9] Rosecrans, J. A., Lovell, R. A., and Freedman, D. X., *Biochem. Pharmacol.*, **16**, 2011 (1967).
[10] Chase, T. N., Breese, G. R., and Kopin, I. J., *Science*, **157**, 1461 (1967).
[11] Marchbanks, R. M., Rosenblatt, F., and O'Brien, R. D., *Science*, **144**, 1135 (1964).
[12] Freedman, D. X., and Giarman, N. J., *Amer. NY Acad. Sci.*, **96**, 98 (1962).
[13] Feldberg, W., and Myers, R. D., *Nature*, **200**, 1325 (1963).
[14] Feldberg, W., and Lotti, V. J., *Brit. J. Pharmacol. Chemother.*, **31**, 152 (1967).
[15] Kulkarni, A. S., *J. Pharmacol. Exp. Therap.*, **157**, 541 (1967).
[16] Shellenberger, M. K., and Elder, J. T., *J. Pharmacol. Exp. Therap.*, **158**, 219 (1967).
[17] Somerville, A. R., and Whittle, B. A., *Brit. J. Pharmacol. Chemother.*, **31**, 120 (1967).
[18] Giarman, N. J., Tanaka, C., Mooney, J., and Atkins, E., *Adv. in Pharmacol.*, **6A**, 307 (1968).
[19] Sheard, M. H., and Aghajanian, G. K., *Nature*, **216**, 495 (1967).

Synaptosomes: Different Populations storing Catecholamines and Gamma-aminobutyric Acid in Homogenates of Rat Brain

It is well established that when brain tissue is homogenized large numbers of nerve terminals pinch off to form intact membrane-bound particles known as "synaptosomes". These can be isolated from brain homogenates by density gradient centrifugation[1–3]. Such particles contain various presumptive transmitter substances such as acetylcholine, noradrenaline (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT)[1–3]. Knowledge of synaptic organization could be greatly advanced if a differential morphology for nerve terminals using various transmitters could be established. Attempts to separate synaptosomes storing different transmitters, however, have met with only limited success[4–6].

Brain slices incubated in physiological media accumulate radioactive catecholamines[7–9] and gamma-aminobutyric acid (GABA)[10] by specific transport mechanisms, so that, if they are accumulated by different neurones, a selective labelling of different types of nerve terminals may be achieved. We have labelled brain slices in this way with various mixtures of catecholamines and GABA labelled with ^{14}C and ^{3}H. By centrifuging homogenates of such slices on sucrose density gradients, we have separated distinct populations of synaptosomes storing catecholamines or GABA. We have so far restricted our attention to slices from two areas of the rat brain, the hypothalamus and the striatum (including the caudate nucleus, the putamen and the globus pallidus)[11]. Those two regions of the brain contain an abundance of catecholamine terminals of two distinct types, the striatal storing dopamine and the hypothalamic storing norepinephrine. Both types of amine terminals can concentrate labelled catecholamines (NE and DA) although the uptake mechanisms in the DA and NE terminals are not identical[7–9].

Sprague–Dawley female rats (weighing 150–180 g) were killed by decapitation. Brains were removed rapidly, chilled and dissected as described previously[11]. Small pieces of tissue (10–15 mg) from striatum or hypothalamus were incubated with shaking in 2 ml. of Krebs–Henseleit