determine the extent of norepinephrine depletion before LSD 25 administration. Brain stems of controls contained 0.58 ± .06 μg/g of tissue, whereas those of rabbits treated with α-MT contained 0.07 ± .03 μg/g. Treatment of animals with only the suspending medium for α-MT exerted no influence on concentrations of norepinephrine in the brain stem.

Our data indicate that both the excitation of the central nervous system and the peripheral sympathomimetic effects caused by LSD in the rabbit are related to the norepinephrine content of the brain. This conclusion is based on the fact that, after depletion of norepinephrine by α-MT, the marked behavioral actions of hyperexcitability, pupillary dilatation, increased motor activity, and so forth, are attenuated or abolished. The hyperthermic action of LSD 25, however, appears to be independent of brain catecholamines, for the temperature changes of controls and animals treated with α-MT in response to treatment with LSD are essentially identical. To our knowledge this is the first instance where it has been possible to separate so clearly the hyperthermic from the other central and peripheral actions of LSD 25. Other workers have employed various agents to antagonize this hyperthermia, but none has obtained the clear dissociation of the behavioral and temperature responses (*8*).

The possibility that LSD 25 exerts its hyperthermic action through a nonadrenergic mechanism is not incompatible with our earlier work in which we postulated a serotonin-like mechanism for the LSD 25 hyperthermia (*9*). The proposal by Feldberg and Myers (*10*) that serotonin may have a possible neurohumoral role in temperature regulation is also consistent with this view.

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Ribonucleic Acid Biosynthesis in Adult and Infant Rat Brain in vitro

Abstract. *The rate of biosynthesis of ribonucleic acid (as judged by the rate of incorporation of uridine into ribonucleic acid) in infant and adult rat brain cortex slices, incubated aerobically in the presence of various substrates, is directly proportional to the adenosine triphosphate concentration. This suggests that the adenosine triphosphate concentration is one of the factors involved in the control of ribonucleic acid biosynthesis in infant and adult rat brain. Acetoacetate or β-hydroxybutyrate is about 70 percent as effective as glucose, with both infant and adult brain, for the promotion of ribonucleic acid biosynthesis, but they are considerably more effective than succinate in infant brain than in adult brain.*

In our experiments on the conditions for optimum rates of biosynthesis of RNA in the isolated brains of immature and adult animals, we have observed that the rate of biosynthesis of RNA in both adult and infant rat brain is directly proportional to the adenosine triphosphate (ATP) concentration in the presence of various substrates.

We prepared slices (0.3 to 0.4 mm thick) from the cerebral hemispheres of Wistar albino rats, using a Stadie-Riggs tissue slicer. The metabolism of the brain slices was investigated by the conventional Warburg manometric technique. The slices were weighed on a torsion balance and transferred to chilled manometric flasks; each flask contained 3 ml of Krebs-Ringer medium composed of 141 m*M* NaCl, 5.0 m*M* KCl, 2.3 m*M* $CaCl_2$, 1.3 m*M* $MgSO_4$, 10 m*M* Na_2HPO_4 (brought to *p*H 7.4 with 1.0*N* HCl), and 5 m*M* glucose. One dorsal and one lateral slice ("first slices"), weighing a total of 50 to 75 mg (wet weight), from the same brain were added to each flask. The glucose in the medium was omitted or replaced by other substrates, as occasion demanded. A solution of radioactive uridine (uridine-2-C^{14}) was placed in the side arm and tipped into the main vessel after its contents had been equilibrated at 37°C for 10 minutes in an atmosphere of oxygen. Incubation with the labeled uridine was carried out for periods of 30 minutes or 1 hour. Measurements of the rates of oxygen consumption were routinely made to ensure that the tissues exhibited their normal respiratory activities. Estimation of the labeled RNA was made by a modification of the technique of Schmidt and Thannhauser (*1*). The slices were removed after incubation, washed twice with ice-cold Krebs-Ringer medium to remove contaminating radioactivity, and homogenized in cold 5 percent trichloroacetic acid. The homogenate was kept at 0°C for 1 hour and centrifuged at 600*g* for 10 minutes; the residue was washed three times with 5 percent trichloroacetic acid to remove acid-soluble radioactive components. The lipids were removed by washing first with 95 percent ethanol and then with a mixture of 95 percent ethanol and ether (3 : 1); the residue was then suspended in 2 ml of 0.2*N* KOH and incubated at 37°C for 18 hours. Perchloric acid (0.05 ml of 70 percent) was then added to precipitate DNA and proteins. The supernatant contained the hydrolyzed RNA whose radioactivity was counted in the liquid scintillation counter. The concentration of ATP was estimated enzymatically by the use of hexokinase and glucose-6-phosphate dehydrogenase. The brain slices, after incubation, were homogenized in 2 ml of 6 percent perchloric acid and centrifuged, and 1.5 ml of the supernatant were neutralized with 5*M* potassium carbonate solution. After centrifugation at 0°C, 0.2 ml of the supernatant was assayed for ATP (*2*) using a flourimetric estimation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) concentration. Sodium acetoacetate solution was made fresh from ethyl acetoacetate by the method of Ljunggren (*3*).

Our results confirm the fact (*4*) that

Table 1. Relation between rates of uridine-2-C^{14} incorporation into RNA of adult and infant rat brain cortex slices and ATP concentration. Incubation was carried out at 37°C in oxygen in Krebs-Ringer–phosphate medium. Uridine (U) was added at concentrations of 8 μM (42.3 mc/mmole) and of 32 μM (10.6 mc/mmole). Substrates were added as sodium salts. Each result is the mean of at least four independent observations ± standard deviation of the mean.

Substrate added (5 mM)	Adult rat					Infant rat (1 to 3 days old)				
		Uridine concentration					Uridine concentration			
		8 μM		32 μM			8 μM		32 μM	
	ATP (nmole/100 mg)	U incorp. (pmole 100 mg^{-1} hr^{-1})	Ratio of U incorp. to ATP conc. (× 10^8)	U incorp. (pmole 100 mg^{-1} hr^{-1})	Ratio of U incorp. to ATP conc. (× 10^8)	ATP (nmole/100 mg)	U incorp. (pmole 100 mg^{-1} hr^{-1})	Ratio of U incorp. to ATP conc. (× 10^8)	U incorp. (pmole 100 mg^{-1} hr^{-1})	Ratio of U incorp. to ATP conc. (× 10^8)
None	62 ± 2	50 ± 6	0.8	105 ± 10	1.7	51 ± 3	177 ± 12	3.5	375 ± 55	7.4
Glucose	162 ± 16	120 ± 2	0.7	235 ± 12	1.5	174 ± 5	550 ± 58	3.2	1425 ± 28	8.2
Acetoacetate	118 ± 8	86 ± 2	0.7	170 ± 3	1.5	115 ± 5	443 ± 35	3.8	845 ± 18	7.3
β-Hydroxybutyrate	98 ± 2	80 ± 6	0.8	140 ± 5	1.4	115 ± 5	428 ± 22	3.7	930 ± 20	8.1
Succinate	118 ± 6	76 ± 8	0.6	165 ± 8	1.4	65 ± 2	239 ± 6	3.7	460 ± 12	7.0

the rate of RNA biosynthesis (as judged by the rate of uridine incorporation into RNA) in infant animal brain exceeds that in the adult brain (Table 1). The rate of RNA formation varies according to the oxidizable substrate present and is approximately proportional to the ATP concentration. This is the case at the two concentrations of uridine-2-C^{14} (8 and 32 μM) tested. The rate of incorporation of uridine into RNA is linear over the time period studied, the amount incorporated in 30 minutes of incubation being half that found after 60 minutes of incubation. After a 1-hour aerobic incubation in presence of glucose, the amount of labeled uridine incorporated into RNA is 0.5 percent of the isotope present in the medium containing 8 μM uridine and 0.24 percent of that present in the medium containing 32 μM uridine.

The proportionality existing between the rate of RNA biosynthesis and the ATP concentration, in both immature and mature rat brains, suggests that the cell content of ATP may, under our experimental conditions, be one of the regulating factors involved in RNA biosynthesis.

The values for the concentration of ATP in the cells from both immature and mature rat brains (Table 1) after aerobic incubation for 1 hour in the presence of glucose closely approximate the values found by Mandel and Edel-Harth (*5*) for the contents of ATP in the brains of albino rats killed by immersion of the whole animal in liquid nitrogen. The concentration of ATP in the immature wet brain tissue is about the same as that in the adult wet brain tissue. If calculations are made, however, in terms of the dry weight of tissue, the ATP concentration of infant brain is considerably higher than in the adult brain, for the dry weight of infant rat brain is 11.5 percent of the fresh weight, whereas that of adult rat brain is 20 percent of the fresh weight.

The results also show that succinate is much less effective than acetoacetate or β-hydroxybutyrate for the formation of ATP in the infant brain than in the adult brain.

Acetoacetate or β-hydroxybutyrate is about 70 percent as effective as glucose for the promotion of RNA biosynthesis in both immature and adult rat brain. Acetoacetate or β-hydroxybutyrate is, however, much more effective than succinate for the promotion of RNA biosynthesis in the immature rat brain than in the adult rat brain.

The relatively high rate of RNA biosynthesis in infant rat brain as compared with that in the adult rat brain is obviously due to a variety of factors besides the ATP concentration, as the ratio of the rate of uridine incorporation into RNA to the ATP concentration is at least four times higher in infant rat brain than in adult rat brain (Table 1). This ratio of activities is approximately the same at the two levels of uridine concentration investigated.

The rate of uridine penetration into the infant rat brain tissue does not differ, under our experimental conditions, from that into the adult rat brain tissue. Measurements of the accumulation of acid-soluble radioactive components in the brain tissue indicate that the rate of entry of uridine into the brain tissue (both infant and adult) is proportional to the uridine concentration in the medium under the given experimental conditions.

A relevant fact to be borne in mind is that there are considerably higher concentrations of other nucleotides [for example, uridine triphosphate (UTP) and guanosine triphosphate (GTP)] in infant (1 to 2 days) rat brain than in the adult rat brain (*5*). The content of UTP in infant rat brain is 49.5 nmole/100 mg (wet weight) and 18.6 nmole/100 mg (wet weight) in adult rat brain (*5*), approximating to concentrations of 0.56 and 0.23 mM, respectively. These values will scarcely be affected by the uridine concentrations (8 and 32 μM) used in this work. It seems possible that the difference in content of UTP is one of the factors involved in the higher rate of RNA synthesis in infant brain than in adult brain.

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