Sub-cellular Distribution of Serotonin in the Developing Rat Brain

It is well known that the neonatal rat brain contains relatively little serotonin[1,2] and that the level of this amine increases slowly with age. Karki *et al.*[3] have shown that the enzymes mediating the synthesis and oxidation of serotonin are also low in the immature rat brain, and increase thereafter with growth of the animal. The aforementioned authors suggest that the low levels of indolamines in the rat brain at birth can be attributed to a deficiency of the binding or storage system, and are not a reflexion of the insufficiency of the enzyme systems. Schanberg and Giarman[4,5] have shown that endogenous brain serotonin distributes itself between the particulate matter of brain homogenates and the supernatants, and that this relationship is susceptible to alteration by drugs. It was, therefore, of interest to investigate the sub-cellular distribution of serotonin in the developing rat brain and to determine whether it will parallel other maturational changes.

Rats of the Wistar strain, bred in our colony under standard conditions of management and nutrition, were used in this investigation, and were selected at various ages after birth. Animals were decapitated rapidly, the brains minus cerebella removed, weighed and homogenized in unbuffered 0·3 M sucrose containing tranylcypramine (1 μmole). All procedures were carried out at 4° C. Sufficient brains were pooled to give a homogenate dilution of 20 per cent (w/v). Duplicate aliquots of the homogenates were centrifuged for 30 min in a No. 40 rotor of the model *L* Spinco ultracentrifuge. The high-speed residue and supernatant were extracted separately for their serotonin content by the method of Shore and Olin[6], as modified by Mead and Finger[7] and assayed in concentrated hydrochloric acid as described by Bogdanski *et al.*[8]. All results are averages of six or more individual determinations, and represent 10–30 animals, depending on the age group.

Table 1. Wet Weights and Serotonin Content of the Developing Rat Brain

Age (days)	Brain wet weight–cerebellum (g)	Percentage of adult brain wet weight–cerebellum	5-HT (γ/g)	Brain 5-HT γ/gm as per cent of adult levels	Percentage of total 5-HT in residue	Ratio brain wt. 5-HT content
1	0·26	17·1	0·26	39·3	72·4	1·0
2	0·26	17·2	0·27	41·6	72·0	1·05
4	0·43	28·6	0·31	47·3	73·5	1·4
10	0·68	45·5	0·30	46·4	77·8	2·3
12	0·85	56·3	0·36	55·3	77·5	2·4
22	1·03	68·6	0·37	56·9	73·2	2·8
31	1·13	75·3	0·50	76·9	78·4	2·3
33	1·32	88·0	0·51	78·9	72·0	2·5
Adult	1·5	—	0·65	—	74·5	2·3

The weight of brains and the amine content of rat brains at various stages of development (Table 1) were obtained without the cerebellum. It is apparent that the rat brain at one day of age contains 39 per cent of the adult amine content, but only 17 per cent of the adult weight. The values for 5-HT content in the developing brain are in agreement with those quoted by Karki *et al.*[3]. At 1 day post-natally, the ratio of brain weight to serotonin is unity, and increases to 2·3 by 10 days. Thereafter, this ratio is approximately constant, suggesting that both wet weight and serotonin-levels are maturing at equal rates.

A number of investigators[4,9] have consistently observed that the bulk of endogenous brain serotonin (75 per cent) is associated with the high-speed residue. Our results show that homogenates prepared from rat brains at all ages from 1 day post-natally yield essentially an adult subcellular distribution, in that in excess of 70 per cent is recovered in the particulate fraction. The concentration of a number of enzymes, such as succinic dehydrogenase[10], cholinesterase[11], and glutamic acid decarboxylase[12,13], is low in the immature rat brain and increases slowly to adult levels. Typical adult electrical activity can only be recorded from the rat cortex at 2 weeks of age[14], and it has been suggested that mature bioelectric activity depends on completion of synaptic connexions in the cortex[15,16].

The suggestion has been made by Karki[3] that the immature rat brain cannot bind serotonin as efficiently as it does at maturity, and a similar phenomenon has been observed by Glowinski *et al.*[17] for the binding of circulating norepinephrine by the developing heart. Though the immature brain may be deficient in its ability to store serotonin, such differences are not evident in the present results on the sub-cellular distribution. It can only be suggested that if specific cellular components are responsible for the binding of small quantities of endogenous amine, then these components do not exhibit maturational changes which are qualitative in nature. It is tempting to speculate that the *in vivo* binding of serotonin might correlate with the maturation of synaptic vesicles, and also possibly to the biosynthesis of gangliosides. Possibly the cellular components responsible for the binding of serotonin are present at birth in the rat brain and mature at the same rates as the wet weight, and the total amine storage capacity, thus resulting in an essentially constant sub-cellular distribution.

We thank Dr. Williamina A. Himwich for her advice in the preparation of this paper.

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Oscillation in Enzyme Reactions

The widespread existence of periodic phenomena in living organisms is well known. These events, like all other material phenomena in living systems, should ultimately be considered in terms of the individual chemical reactions occurring during the life process. Since chemical reactions occurring in biological systems are catalysed by enzymes, it was the purpose of our investigation to examine the possibility of oscillatory behaviour in enzyme-catalysed reactions.

The mathematical treatment of the differential equations arising in chemical kinetic problems is often impeded by the fact that the exact analytic solution of the equations is difficult, if not impossible, to obtain. Nevertheless, Lotka[1] in 1910 proposed an autocatalytic system wherein periodicity is clearly possible, and in 1921 Bray[2] illustrated experimentally a chemical system wherein periodicity is actually observed.

Difficulties attending the mathematical treatment of the differential equations arising in enzyme kinetic problems have served as a severe impedance to the development of enzyme kinetic theory. However, Christiansen[3] and Ozawa[4] obtained analytical results indicating that oscillations could occur in certain types of enzyme-catalysed reactions, provided certain conditions are met. Since then, Anderson[5], Chance[6] and Sbnoll[7] have