mikroskopischen Aufnahmen vesikuläre Strukturen darstellen. Die langsamer sedimentierende Komponente (85S) hat einen kleineren Durchmesser (507 Å) und ist weniger dicht (1.16 g/cm³) als die schneller sedimentierende Komponente mit einem durchschnittlichen Durchmesser von 609 Å und einer Dichte von 1.20 g/cm³. Die Dekapazitationsaktivität in Samenflüssigkeit kommt beiden zu und nicht nur wie bis jetzt angenommen wurde, der 85S Komponente. Zwei verschiedene Vesikeltypen wurden auch in der Samenflüssigkeit von Mensch und Stier gefunden.

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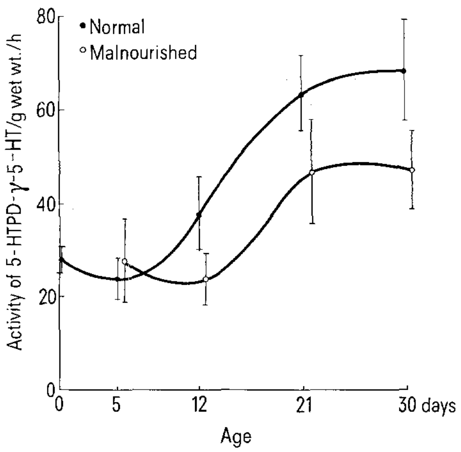
Developmental Pattern of the Serotonin Synthesizing Enzyme in the Brain of Postnatally Malnourished Rats

Study of the metabolism of brain biogenic amines in animals malnourished from birth represents an opportunity to evaluate the effects of malnutrition on a biochemical parameter closely related to neuronal function during development and maturity. 5-Hydroxy-tryptamine (5-HT) acts in the brain as a neurotransmitter and seems to participate in the integration and control of psychoemotional behaviour[1-6]. We have therefore considered it of importance to study the metabolism of 5-HT in the brain of normal and malnourished developing rats. The activity of the brain enzyme 5-Hydroxyrtyptophane-Decarboxylase (5-HTPD) which synthesizes 5-HT was measured as well as the concentration of this amine. The results will be outlined here.

Methods. Albino rats, originally Wistar strain, were malnourished from birth with the method used by Widdowson and Kennedy[7]. Newborn rats were redistributed within 12 h of birth into litters of 6 and 16 per mother, the former were considered controls and the latter malnourished. On day 21 the young were separated from the mother in individual cages and fed Purina chow ad libitum (control group) or 50% of the normal caloric requirements (malnourished group). Water was offered ad libitum to all animals. Body and brain weight curves were determined in both groups. Animals were killed by decapitation on days, 5, 12, 21, and 30, the brain was dissected (cerebellum, pineal and olfactory bulbs were not included), weighed, and homogenized in 0.25 *M* cold sucrose or 0.1 *N* HCl. For the estimation of 5-HTPD-activity the method of Kuntzman et al.[8], was used. For measuring serotonin concentration, the method described by Snyder et al.[9] was employed.

Results and discussion. Deficits in body and brain weight in malnourished animals were, at 30 days of age, of the order of 40% and 15% respectively as compared with the controls. The developmental curve of the activity of 5-HTPD in the whole brain of rats from both groups studied is shown in the Figure. In the control group the enzyme curve shows a decrease in activity between birth and the 5th day, increasing thereafter up to the 21st day, with a lower rate of increase between days 21 and 30. 5-HTPD activity in the brain of malnourished rats showed a tendency to be lower than in controls, being significantly lower on day 21 ($p < 0.05$), calculated on the basis of dry weight (Table I). The decrease in enzyme activity observed on the 5th day in the controls was observed on the 12 day in the brain of malnourished



Activity of 5-Hydroxytryptophane-decarboxylase in the brain of rats malnourished from birth and normal controls. Each point represents the mean activity from 3 to 14 samples, from at least 3 different litters ± S.E. The enzyme activity is expressed as µg of 5-HT/g of wet weight/1 h.

Table I. Activity of 5-Hydroxytryptophane-Decarboxylase (µg of 5-HT/g dry weight/1 h ± S.E.)

Age	12 days	21 days[a]
Normals	553.9 ± 62.4	1061 ± 90.4
Malnourished	369.8 ± 126.9	821.7 ± 65.2

[a] p 0.05.

Table II. Mean concentrations of 5-Hydroxytryptamine, ng/g wet weight, in the brain of Normal (N) and Malnourished (M) rats ± S.E.

	Age in days 0	5	12	21	Adults
N	345 ± 7.7	138 ± 20.0	277 ± 10.7	352 ± 42.0	523 ± 33.1
M	—	151 ± 12.6	345 ± 30.2	307 ± 66.2	—

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animals. This possibly represents a retardation of biochemical brain development of the underfed animals. It is interesting that significant differences in 5-HTPD activity appreared only on day 21. At this age the 5-HTPD activity in the malnourished brain is equivalent to the activity present in the controls in the 2nd week, representing also an immature biochemical feature. LOIZOU[10,11] reported that 5-HT content of neurone bodies and terminals in the brain of the rat attained adult values by the 3rd week. It is possible that in this period the metabolic changes induced by early malnutrition are more striking. SHOEMAKER et al.[12], studying the offspring of rats fed a low protein diet during pregnancy, found a low content of norepinephrine and dopamine in the brain on the 24th postnatal day. Our results on 5-HTPD activity also suggest a modification of the brain biogenic amines metabolism in the 3rd week of postnatal life in early malnourished rats. EAYRS et al.[13], found impoverishment of the neuropil network which normally matures from day 20th to day 25th, in the brain of early malnourished rats. The possible metabolic changes in this period could reflect alterations of synaptic maturation and function.

The concentration of the brain 5-HT was not significantly different between the 2 experimental groups at the various ages studied, although a tendency to lower values in the malnourished group on day 21 was noticed (Table II). It is possible that normal concentrations of the brain 5-HT could be supplied even by a lower activity of 5-HTPD, although low utilization or a modification in the catabolism of the amine should be also considered. On the other hand, considering the brain as a whole, real differences in 5-HT levels in the malnourished could be masked. In more discrete and specific regions of the brain, in which serotonin concentrations are normally different, more striking changes in its metabolism might be found in the developping malnourished rat. Further studies concerning 5-HTPD activity, 5-HT, and noradrenaline levels in different brain regions in normal and early underfed rats are planned.

Résumé. Dans le cerveau de rats souffrant de malnutrition depuis la naissance, on a déterminé l'activité de l'enzyme 5-hydroxytryptophane-déscarboxylase pendant le premier mois du développement. Une différence significative a été observée le 21ème jour par rapport aux contrôles. En même temps, la concentration endogène de 5-HT ne présenta qu'une tendence à diminuer.

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Gramicidin S Analogs Containing N-Methylleucine in Place of Leucine

For the conformation of gramicidin S (GS), several models have been proposed. A possible model, which was first suggested by HODGKIN and OUGHTON[1], is the intramolecular antiparallel β-form with 4 hydrogen bondings between the valyl and the leucyl residues[2-4]. In order to investigate to what extent these hydrogen bondings will stabilize the conformation, we synthesized 2 analogs of GS, lacking 1 or 2 of the 4 intramolecular hydrogen bondings.

Material and method[5]. Boc-Orn(Z)-OH was coupled with H-MeLeu-OMe by the use of the water-soluble carbodiimide[6] to yield Boc-Orn(Z)-MeLeu-OMe (I) in 50% yield. Saponification of I gave the corresponding acid (II) as fine crystals. Boc-Orn(Z)-MeLeu-D-Phe-Pro-OEt (III) was obtained from II and H-D-Phe-Pro-OEt in a nearly quantitative yield using dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole[7]. After removal of the Boc group of III, the resulting tetrapeptide ester was coupled with Boc-Val-ONSu to yield Boc-Val-Orn(Z)-MeLeu-D-Phe-Pro-OEt (IV), in 80% yield, which was converted to the corresponding acid (V) by saponification and subsequently to H-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH (VI) by the action of hydrogen chloride in ethyl acetate. Condensation of the azide derived from Boc-Val-Orn(Z)-Leu-D-Phe-Pro-$NHNH_2$[8] with VI gave Boc-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH (VII) in 53% yield. VII was then transformed to the Boc-decapeptide N-hydroxysuccinimide ester (VIII) by the reaction of N-hydroxysuccinimide and the water-soluble carbodiimide. After removal of the Boc group of VIII with trifluoroacetic acid, the decapeptide ester

Antibacterial activity of the compounds (minimum inhibitory concentration, μg/ml)

Strain	Gramicidin S	[MeLeu[3]]-Gramicidin S (X)	[Di-MeLeu[3,3']]-Gramicidin S (XV)
Staphylococcus aureus	6.25	6.25	6.25
Bacillus subtilis	3.12	3.12	3.12
Escherichia coli	100	50	50
Shigella flexneri	100	100	100
Candida albicans	50	50	25

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