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CONCENTRATION OF 5-HYDROXYTRYPTAMINE IN THE SMALL INTESTINE AND PLATELETS OF THE DEVELOPING GUINEA PIG

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Abstract—In guinea-pigs, 5-hydroxytryptamine first appeared in the small intestine in measurable amounts after 30–40 days of intra-uterine life. The rise in concentration of the 5-hydroxytryptamine in the platelets followed the rise in the intestine. These results support the view that platelet 5-hydroxytryptamine is derived from the intestine. They are compared with previous observations on the concentration of 5-hydroxytryptamine in the developing guinea-pig brain.

SMITH, Stacey and Young[1] found that at birth the foetal brain of both the guinea pig and rat had considerable 5-hydroxytryptophan decarboxylase activity. The concentration of 5-hydroxytryptamine was about 70 per cent of the adult level in the guinea pig, but only 40 per cent in the rat; these findings have been confirmed by Karki, Kuntzman and Brodie.[2] Nachmias[3] found very low concentrations of 5-hydroxytryptamine in the brains of newborn rats and similar results were obtained by Pepeu and Giarman[4] for the newborn rabbit brain.

In view of the well known maturity and activity of the guinea pig at birth in comparison with the helplessness of the newborn rat and rabbit, it seemed possible that 5-hydroxytryptamine storage in the brain might be an indication of maturity. The present study was carried out to determine whether this sign of maturity could be extended to the platelets and to the small intestine. Low concentration of 5-hydroxytryptamine have been found in human cord blood at term by Norris and Stacey,[5] and in the newborn rabbit by Pepeu and Giarman[4] who, however, found higher concentrations in the foetal goat than in the adult.

Because of previous observations, a comparison has also been made of the 5-hydroxytryptamine concentrations in the brain and in the small intestine of litter mate virgin and pregnant guinea pigs.

MATERIAL AND PROCEDURE

A. Concentrations of 5-hydroxytryptamine in the gut and platelets of the developing young

The observations were made on 17 guinea pigs and their foetuses at gestational ages ranging from the thirtieth to the sixty second day of pregnancy (term is 67 days gestation). The foetuses were weighed and the approximate age estimated from the

values given by Draper[6] and Bell[7]; the weights ranged from 32–62 g. 16 guinea pigs, 3–40 days of age and 98–520 g weight, were also studied.

The maternal and newborn animals were lightly anaesthetized with pentobarbitone sodium (20–30 mg/kilo) given into the brachial vein under local procaine injection. Blood was collected from the adults and larger newborns from a polythene catheter in the carotid artery, straight into ice cold anticoagulant (1% sodium edetate in 0·7% NaCl) in siliconed tubes: it was drawn into siliconed syringes from the umbilical vein in the foetuses and collected by cardiac puncture in the smaller newborns. Approximately 9 vol. blood were added to 1 vol. anticoagulant. The small intestines were removed immediately and the contents squeezed out, gently; the empty gut was slit longitudinally, washed in cooled 0·9% NaCl and weighed. A representative aliquot was homogenized in 0·1 N HCl.

B. Concentrations of 5-hydroxytryptamine in the non-pregnant and pregnant maternal brain and gut

Seven young guinea pigs were studied at the end of their first pregnancy; seven litter mate virgins were used as controls. The animals were killed by a blow on the head because of the finding that barbiturate anesthesia elevates brain 5-hydroxytryptamine concentration.[8, 9] No blood samples were taken, but the brain and small intestines were removed immediately. The gut was treated as described above and the brain was weighed and homogenized in 0·1 N HCl.

METHODS

Platelet counts. Whole blood platelet counts were made on a 1/20 dilution of the blood in 1% ammonium oxalate, and corrected for the dilution factors. Platelet rich plasma was obtained by centrifuging the blood at 4°, and 800 rev/min (160 *g*) for 20 min; a platelet count was made on the supernatant and the platelets separated from the plasma by centrifuging 2 ml for 30 min at 3,000 rev/min (2,200 *g*). The platelet-free supernatant was poured off and discarded, the tubes allowed to drain and wiped free of any remaining plasma with filter paper. Duplicates were prepared where possible. The platelets were of uniform size in the adult blood but it was most noticeable that they were of uneven size in the foetal and neonatal blood. As the smaller platelets were lost in the first centrifuging, the count in the foetel and neonatal plasma was usually lower than in the whole blood.

Estimation of 5-hydroxytryptamine. Blood. The platelet pellet was mixed thoroughly with 0·5 ml water and the 5-hydroxytryptamine estimated fluorimetrically by the method of Bogdanski, Pletscher, Brodie and Udenfriend.[10]

Intestine and brain. Estimations were made in duplicate on aliquots of the homogenate by the same method.[10] In all cases internal standards were used: this is essential as the extraction rates from tissues of animals of different ages differ.

RESULTS

A. Concentration of 5-hydroxytryptamine in small intestine and in platelets during development

These results are shown in Figs. 1 and 2. Very low concentrations of 5-hydroxytryptamine were found in the small intestine and platelets of the younger foetuses examined (mid term) but there was a steady rise during the second half of pregnancy and in the

the newborn period. In the intestine, the rise started about the 40th day of gestation and preceded the rise in platelet 5-hydroxytryptamine concentration by 10–15 days. The range of the values was much greater for the neonates than for either the foetuses or the adults. In intra-uterine life, while the concentration of 5-hydroxytryptamine in gut and platelets was rising rapidly, gut growth was slow and the platelet coun already nearly equal to the adult value. By contrast, in early neonatal life slower changes in concentration occurred in both platelets and gut although the growth of the latter was rapid.

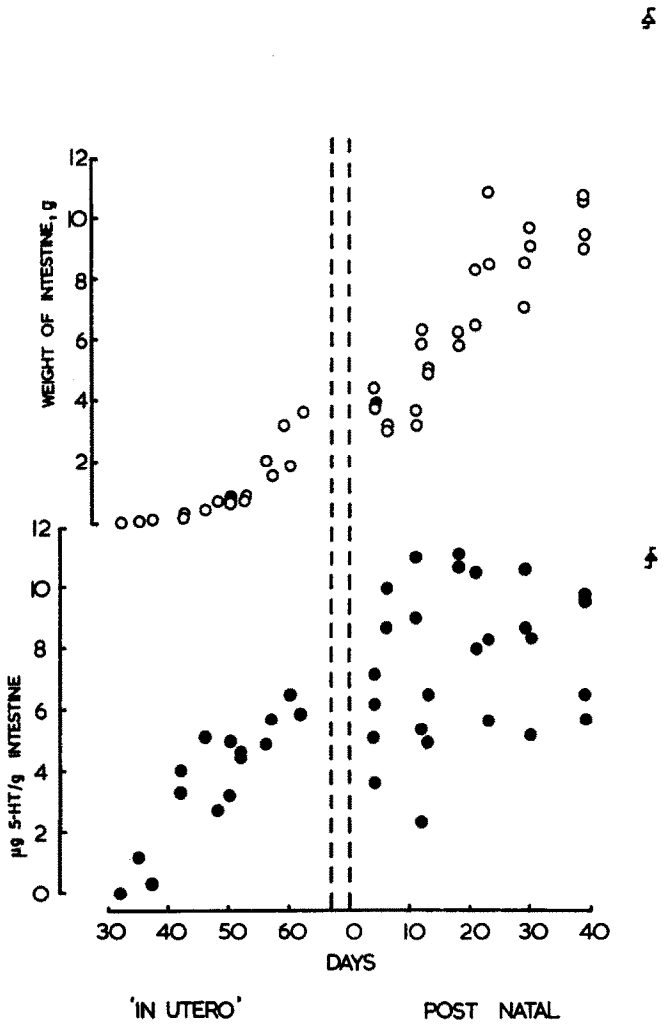

FIG. 1. Small intestine weight (○) and 5-hydroxytryptamine concentration (●) "in utero" andearly life: a comparison with the mean maternal values (triangles ± S.E.M.).

B. Brain and small intestine 5-hydroxytryptamine in the pregnant and non-pregnant

The concentrations of 5-hydroxytryptamine in the brain and small intestine of pregnant guinea pigs were higher than in their non-pregnant litter mates in six out of seven

pairs of animals. Means are recorded in Table 1 where it is shown that the differences are small, and not significant at the 5 per cent level. The total weight of the small intestine of the non-pregnant animals was in all pairs considerably greater than that of the pregnant animal.

DISCUSSION

The first appearance of 5-hydroxytryptamine in the small intestine and in the platelets occurs half way through the gestation period, at which time the concentration of 5-hydroxytryptamine in the foetal brain is already half that found in the adult.[1] Thereafter the concentration in the gut rises more rapidly than that in the brain till at birth, both reach a level about 60 per cent of the adult level. How far the delay in

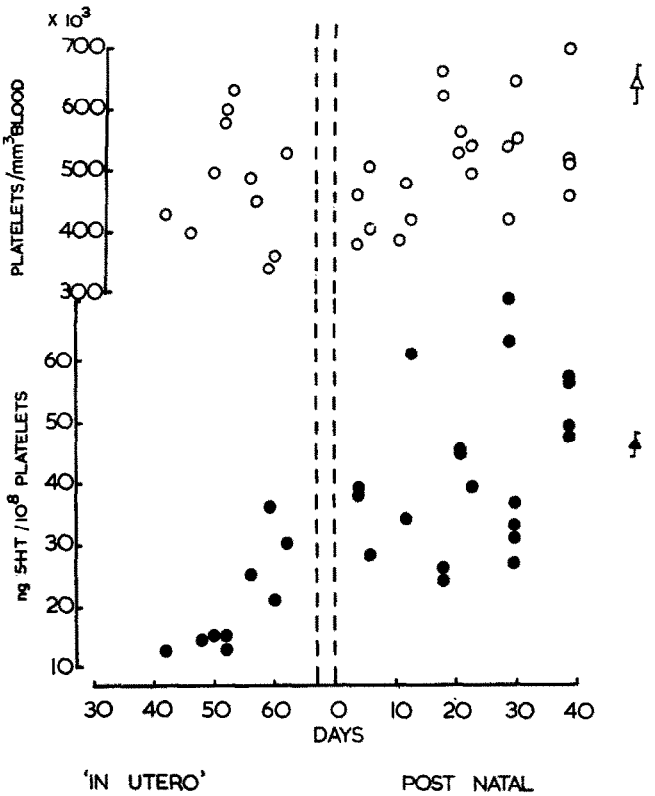

FIG. 2. Platelet counts (○) and 5-hydroxytryptamine concentration (●) "in utero" and early life: a comparison with the mean maternal values (triangles ± S.E.M.).

the appearance of 5-hydroxytryptamine in the small intestine is due to a delay in synthesis and how far it awaits the development of storage capacity, is not known. It would be interesting to be able to correlate the appearance of 5-hydroxytryptamine in the intestine with the development of argentaffin cells. Histological observations on the guinea pig do not appear to have been made, but in calves Faustini[11] found that

TABLE 1. COMPARISON OF THE 5-HT CONTENT OF THE BRAINS AND SMALL INTESTINES OF 7 PAIRS OF PREGANT AND NON-PREGNANT LITTER MATE GUINEA PIGS

(The mean values for the two groups and the mean differences between pairs of litter mates are given, together with the standard error of the mean).

	Pregnant	Non-pregnant	Mean Difference	P
Body wt. (g)	887	748		
Brain				
wt. (g)	3·93	4·19	0·27 ± 0·13	0·1
μg 5-HT/g	0·420	0·375	0·045 ± 0·022	>0·05
total μg 5-HT	1·65	1·56	0·08 ± 0·11	>0·10
Small intestine				
wt. (g)	10·8	13·1	2·38 ± 0·58	<0·01
μg 5-HT/g	11·4	10·0	1·39 ± 1·03	>0·10
total μg 5-HT	124	131	7·3 ± 10·3	>0·10

5-hydroxytryptamine and argentaffin cells appeared together at the 5th to 6th week of intra-uterine life and attained their maximum together at the 11th to 13th week; Cole and McKalen[12] report the appearance of argentaffin cells in the human foetus at between the 6th and 12th week.

Even in the youngest foetuses in which platelet counts were made, those of 40–50 days gestation, the numbers of platelets were similar to those in adults; but only towards the very end of pregnancy did they begin to contain appreciable quantities of 5-hydroxytryptamine. If, as is probable, platelet 5-hydroxytryptamine is derived from the intestine[13] the later appearance of 5-hydroxytryptamine in the platelets is to be expected. However, there is the further possibility that the platelets of the very young foetus may be unable to concentrate and store 5-hydroxytryptamine. Low platelet 5-hydroxytryptamine concentrations have also been observed in the newborn rabbit[4] and in human cord blood; in the latter case the low values are not due to storage failure since the capacity of the platelets to take up 5-hydroxytryptamine *in vitro* is equal to that of adult platelets.[5]

A careful comparison of litter mates has confirmed our previous finding that at term the 5-hydroxytryptamine content of the brain of pregnant guinea pigs is usually slightly higher than that of the non-pregnant; however the difference was not statistically significant nor was there a significant difference in the amount of 5-hydroxytryptamine in the small intestine of the two groups. The lighter small intestines of the pregnant animals may be due to diminished growth in these animals.

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