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# EXPERIMENTAL MODEL WITH AN ADDITIONAL SOURCE OF ENDOGENOUS SEROTONIN

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The only experimental method of producing a relative selective increase in the serotonin level in the body at present is by administering precursors for its synthesis. An important disadvantage of the method is the short duration of the rise in the serotonin level and the impossibility of differentiating between its central and peripheral effects [9].

In this paper we describe an experimental model with an additional source of synthesis of endogenous serotonin (ASES model). It is based on the ability of grafts of the mouse embryonic gastrointestinal tract to survive when transplanted subcutaneously into adult syngeneic mice, as several workers have discovered [2, 4], and subsequently to develop and, as we ourselves have shown [3], to produce serotonin.

## EXPERIMENTAL METHOD

On the 18th-21st day of pregnancy BALB/c mice were decapitated, the fetuses were removed under sterile conditions from the uterus in the cold, and the stomach and adjacent portion of the duodenum, which contain the highest concentration of enterochromaffin (EC) cells, which produce and accumulate serotonin [10], in mice, were isolated. The graft was transplanted subcutaneously into anesthetized adult male mice (aged 2.5-3 months) of the same line. The animals were killed by quick decapitation under standard conditions 30-40 days later, when organ-like formations (cysts) had reached their maximal size.

The serotonin concentration was determined fluorometrically [6] in the blood and also in the brain stem and grafts. The principal metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), was determined in the brain stem by the same method.

Tissue for morphological investigation was fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Sections were stained with azure-eosin and the PAS reaction. To identify EC cells and other endocrine cells a combination of staining with Fast Garnet and lead hematoxylin [1] was used. Serotonin in the cells was detected by the Falck-Hillarp fluorescence histochemical method [8].

Male BALB/c mice of the same age (3.5-4 months), both intact and undergoing a mock operation, served as the control for determination of the blood and brain serotonin levels; similar mice at the age of 1-1.5 months were used to investigate the morphology of the stom-

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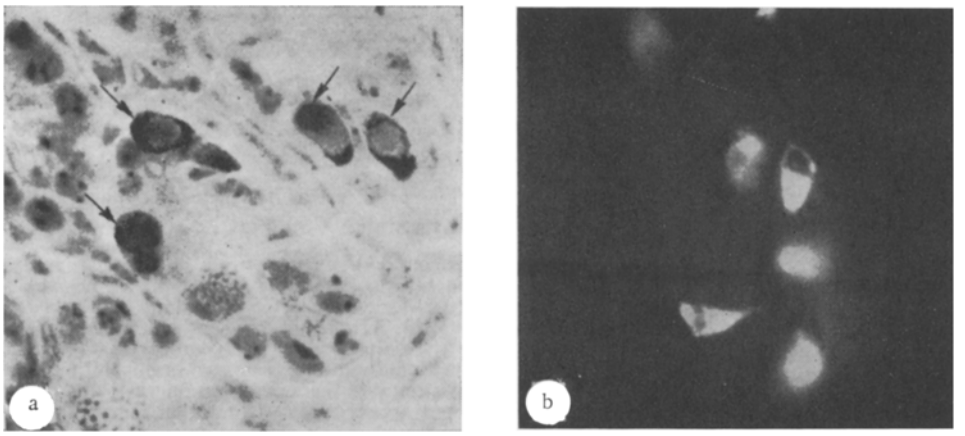

Fig. 1. EC cells in crypts of grafted and developing mouse small intestine: a) stained with Fast Garnet and lead hematoxylin; b) stained by Falck–Hillarp method. 800 ×.

ach and to determine its serotonin content for comparison with the corresponding parameters during postnatal development of the grafts, over a period of 30–40 days.

## EXPERIMENTAL RESULTS

The grafted segments of fetal gastrointestinal tract 30–40 days after the operation consisted of organ-like formations penetrated by numerous blood vessels. Morphological investigation of the mucous membrane of the developing graft revealed various types of cells characteristic of the corresponding regions of the stomach and duodenum of intact animals: chief, parietal, and mucoid cells, enterocytes with brush border, goblet cells, Paneth cells, endocrine cells and, in particular, EC cells (Fig. 1A). EC cells were found in the mucous membrane of the whole stomach, but most of them were located in the juxtapyloric part, and also in the duodenum. In the stomach they were distributed mainly in the basal portions of the glands, and in the intestine in the crypts, in agreement with their distribution in the control mice.

Mast cells, the granules of which in mice also contain serotonin [11], were found in the upper portions of the tunica propria of the mucuous membrane of the body of the stomach. They were fewer in number than in the stomachs of the intact mice aged 1 month. Intensive yellow fluorescence of granules of EC cells (Fig. 1b) and mast cells, characteristic of serotonin, was revealed by the Falck–Hillarp fluorescence-histochemical method.

A fairly high serotonin level, on average about half of its concentration in the stomach of intact mice aged 30–40 days, was found by a biochemical method in the graft (Table 1). Considerable fluctuations of the serotonin concentration were noted, from 1 to 7 μg/g; these were evidently attributable to differences in the degree of development and blood supply of the grafts. In about 30% of the experiments the grafts did not develop.

The biochemical and histomorphological investigations thus revealed that organ-like formations developing from embryonic stomachs can serve as a source of endogenous serotonin: EC cells synthesizing serotonin were found in them, and considerable quantities of serotonin, comparable with its concentration in intact stomachs, were identified.

The existence of an additional source of serotonin synthesis in the recipient animal led to a marked increase in the concentration of this amine in the blood of the mice (Table 1). The mean blood serotonin level in the ASES model was raised by 40% compared with that in intact animals and animals undergoing a mock operation. (These two control groups did not differ significantly — $P > 0.05$ — and the values were therefore pooled.)

A long-term rise in the blood serotonin concentration on average by 40% was shown not to lead to any change in its level in the brain stem (Table 1). There was likewise no change in the 5-HIAA concentration (1.03 ± 0.04 μg/g in the control and 1.01 ± 0.03 μg/g in mice of the ASES model; $P > 0.05$). Only when the serotonin concentration in the blood of mice with grafts of the gastrointestinal tract was on average 60% higher was its concentration in the brain also found to be higher than that of the control animals (0.85 ± 0.03 and 0.69 ± 0.05

TABLE 1. Serotonin Concentration in Blood (in μg/ml), Stomach Grafts, and Brain (in μg/g) of Mice in ASES Model (M ± m)

| Tissue | Serotonin | | P |
|---|---|---|---|
| | ASES model | control | |
| Stomach grafts | 3,97±0,07 (14) | 7,85±0,86 (10) | <0,05 |
| Blood | 1,89±0,08 (20) | 1,35±0,05 (24) | <0,001 |
| Brain | 0,81±0,05 (13) | 0,73±0,04 (19) | >0,05 |

Legend. Stomachs of mice aged 1 month, i.e., an age similar to the period of postnatal development of the graft, were used as the control for stomach grafts in the ASES model.

μg/g respectively; $P < 0.05$). However, there was no change in the 5-HIAA concentration under these circumstances (1.29 ± 0.30 and 1.21 ± 0.18 μg/g; $P > 0.05$).

The blood–brain barrier is known to be impermeable to serotonin [5]. Even during a long-term rise of 40% in the blood serotonin level, none penetrates into the brain. With a similar rise in the blood serotonin concentration, the ASES model is correspondingly a model of a selectively, peripherally raised serotonin level. If higher concentrations of serotonin were present in the blood, it would perhaps penetrate into the brain tissue. However, the possibility cannot be ruled out that the small rise in the serotonin level which we found in the brain was the result of its raised concentration in the blood of the cerebral vessels, for no changes were found in the 5-HIAA concentration in the brain under these circumstances. Meanwhile an experimental increase in the serotonin concentration in the brain is known to lead to intensification of oxidative deamination processes with the formation of 5-HIAA [7].

A prolonged rise in the endogenous serotonin level in the blood produced by an additional source of its synthesis can be used to study the role of serotonin in the peripheral stages of regulation of various physiological systems and to reveal its effects arising during a chronic rise in its blood concentration, which is observed in some pathological states. In view of individual fluctuations in the degree of elevation of the blood serotonin level, when the ASES model is used its blood concentration must be determined in all the experimental animals.

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