Results. Effect of melatonin. Melatonin decreased ^{14}C-glucose oxidation to $^{14}CO_2$ at concentration of 10 µg/ml Simultaneously, it decreased its incorporation into glyceride-glycerol fraction at a level of 5 µg/ml and into fatty acids at concentration of 1 µg/ml (Figures 1–3).

Effect of serotonin. Serotonin did not have any statistically significant effect on glucose oxdiation or fatty acid synthesis at concentrations even higher than 10 µg/ml. A decrease in glucose incorporation into the glyceride-glycerol fraction was noted at the 10 µg level (Figures 1–3).

Discussion. Although melatonin and serotonin are structural analogues, their effects on glucose metabolism appear to be different. Melatonin decreased ^{14}C-glucose oxidation while serotonin was inactive. Melatonin decreased ^{14}C-glucose incorporation into glyceride-glycerol fractions and serotonin showed qualitatively similar effects. Melatonin decreased ^{14}C-fatty acid synthesis while serotonin did not show similar effects. These data indicate that melatonin may have peripheral effects, other than those described previously[2,3], and its peripheral inhibitory effects[10] may partly be mediated by its effects on glucose metabolism. The physiological significance of these observations in regulation of lipid metabolism in vivo is at present uncertain and remains to be elucidated[11].

Résumé. Nous avons étudié les effets de la mélatonine et de la sérotonine sur le métabolisme du ^{14}C-glucose dans le tissu adipeux du lapin. La mélatonine semble inhiber la synthèse des acides gras et l'oxidation du glucose alors que la sérotonine est sans action. La mélatonine et la sérotonine empêchent l'incorporation du ^{14}C-glucose dans la fraction glycéride-glycérol.

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Hormonal Imputs and Brain Tryptophan Metabolism: the Effect of Growth Hormone

A large body of experimental evidence in animals and in the human has accumulated in recent years for the participation of brain monoamines, norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in the neurohormonal control of anterior pituitary (AP) hormones[1].

Much attention has been given to the presence of serotoninergic pathway(s) mediating neuroendocrine regulation of gonadotropins and prolactin secretion. Intraventricular instillation of 5-HT in the rat enhanced prolactin concentration in the peripheral plasma and decreased plasma concentrations of gonadotropins[2]. Systemic administration of 5-HT precursors, l-tryptophan (TP) or 5-hydroxy-L-tryptophan (5-HTP) increased serum prolactin concentrations in both laboratory animal[3] and the human[4]; 5-HTP has also been implicated in the control of growth hormone (GH) release in the human[5], a finding not corroborated by other studies[6].

Studies on the effect of varying endocrine function on 5-HT metabolism have also been reported, though most have been concerned with the effects of adrenal steroids[7–10]. Here we report data on brain 5-HT metabolism in two experimental conditions which have in common a lack of pituitary GH and refer also to the effect of a GH replacement therapy.

Female Sprague-Dawley rats were obtained at weaning, fed a standard laboratory diet ad lib, maintained at 22 ± 2 °C and exposed to 14 h of light each day (06.00–20.00 h). Experiments were performed 15 days following hypophysectomy in 40-day-old rats. Age-matched intact rats were used as controls. On the day of the experiment animals were killed by decapitation between 08.30–09.00 h. Brain was removed, weighed and stored at −20 °C until assayed for TP, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA)[11,12]. Brain concentrations of both TP and 5-HIAA were markedly higher in hypophysectomized rats than in age-matched intact controls, while brain 5-HT levels were comparable in the two groups (Table I). It is generally recognized that the rate of 5-HT formation in the brain is regulated principally by the availability of the amino acid precursor TP, the normal concentration of which in tissues is below the *Km* for the first enzyme in 5-HT biosynthetic pathway, tryptophan hydroxylase[13]. The finding that hypophysectomized rats had elevated brain TP levels, and the reported inability of hypophysectomy to affect brain TP hydroxylase[14], suggest that pituitary ablation may be associated with an increased brain 5-HT metabolism. The observation that brain 5-HIAA concentrations were also increased by hypophysectomy further substantiates this hypothesis. However, an alteration of 5-HIAA efflux rate from the brain cannot be excluded at present.

The effect of hypophysectomy on brain 5-HT metabolism could be ascribed to the deficiency of one or more hormonal product(s) secreted by the AP itself or by a peripheral target gland under AP regulatory control.

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The findings that corticosterone deficiency in the adrenalectomized rat or administration of ACTH to normal animals are associated respectively with a decrease or an increase in brain 5-HT turnover[7–10], seem to rule out the possibility that the effect of hypophysectomy on 5-HT metabolism may be mediated by a lack of adrenal corticoids or ACTH.

Among the several physiological factors whose lack could be responsible for the effect of pituitary ablation on brain indoles, growth hormone deserves particular attention. The possibility that GH may alter 5-HT metabolism through the availability of the 5-HT amino acid precursor, i.e. TP, has to be considered on recalling the profound effect of GH on amino acid metabolism[15].

For testing the role of GH, a group of hypophysectomized rats received i.p. daily for 7 days a dose of 1.0 mg bovine GH (NIH-B-17). After killing, brain was removed and TP, 5-HT and 5-HIAA determined as previously indicated. GH therapy resulted in hypophysectomized rats in a significant reduction of brain TP and 5-HIAA concentrations and did not modify brain 5-HT (Table I). The latter findings suggested GH deficiency to be responsible, at least partially, for the altered brain TP and 5-HT metabolism present in the hypophysectomized rats.

Table I. Brain concentrations of tryptophan (TP), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in hypophysectomized (hypox) and hypox-growth hormone (GH) treated rats

Treatment	Brain		
	TP (μg/g)	5-HT (μg/g)	5-HIAA (μg/g)
Intact controls	3.40 ± 0.19	0.64 ± 0.012	0.39 ± 0.008
Hypox	6.92 ± 0.30 [a]	0.63 ± 0.010	0.65 ± 0.017 [c]
Hypox + GH	4.92 ± 0.20 [b]	0.62 ± 0.017	0.54 ± 0.026 [d]

Hypophysectomy was performed at 26–28 days of age. Animals were maintained for 2 weeks and those with evidence of continuous growth were not used. The completeness of hypophysectomy was determined in each animal at autopsy. GH was administered daily (1.0 mg i.p.) for 7 days. Rats were killed 20 h after the last injection of GH. Administration of GH to hypophysectomized rats induced a significant increase in body weight, but did not increase mean food intake. Each value is the mean of 5 determinations ± the standard error of the mean. [a] $p < 0.001$ vs intact controls; [b] $p < 0.001$ vs hypox; [c] $p < 0.001$ vs intact controls; [d] $p < 0.01$ vs hypox.

Table II. Brain concentrations of TP, 5-HT and 5-HIAA in dwarf Snell-Bag mice (dw/dw) and in heterozygote controls (dw/+)

Treatment	Brain		
	TP (μg/g)	5-HT (μg/g)	5-HIAA (μg/g)
(dw/+)	4.20 ± 0.21	1.15 ± 0.055	0.33 ± 0.020
(dw/dw)	6.40 ± 0.33 [a]	1.15 ± 0.024	0.65 ± 0.026 [c]
(dw/+) + GH	3.87 ± 0.19	1.33 ± 0.093	0.26 ± 0.026 [d]
(dw/dw) + GH	5.57 ± 0.20 [b]	1.30 ± 0.010	0.57 ± 0.014 [e]

The mean food intake/g b.wt. was not significantly different in dwarf mice and controls. GH was administered daily (200 μg i.p.) for 7 days. Mice were killed 20 h after the last injection of GH. Administration of GH resulted in a slight body weight increase in dwarf mice, but did not increase body weight of controls over that present in untreated mice. Mean food intake in both dwarf mice and controls was not changed by GH treatment. Each value is the mean of at least 6 determinations ± the standard error of the mean. [a] $p < 0.001$ vs (dw/+); [b] $p < 0.05$ vs (dw/dw); [c] $p < 0.001$ vs (dw/+); [d] $p < 0.02$ vs (dw/+); [e] $p < 0.02$ vs (dw/dw).

A more proper evaluation of GH role was made by studying brain TP metabolism in genetically dwarf mice, which are characterized by a selective deficiency in the secretion of GH and prolactin from the anterior pituitary[16].

Female dwarf mice (dw/dw), 4 weeks old, of the Snell-Bag strain, descendants of a colony originally obtained by the Jackson Laboratories, Bar Harbor, Me, were used. Age-matched heterozygote mice (dw/+) were used as controls. Similarly to the hypohysectomized rats, GH-deficient mice had brain TP and 5-HIAA concentrations markedly higher than controls. A GH treatment (200 μg i.p. daily, for 1 week) decreased TP and 5-HIAA levels both in dwarf mice and controls. In no one of the above experimental conditions was there a change in brain 5-HT concentrations (Table II).

Although, as stated above, dwarf mice lack also prolactin[16], the latter hormone does not seem to play a significant role in affecting brain 5-HT metabolism. A prolactin replacement therapy did not change significantly brain TP levels in both dwarf mice and hypophysectomized rats.

Collectively, these data demonstrate that a deficiency in circulating pituitary hormone(s), as present in the hypophysectomized rat or dwarf mouse, can affect the brain metabolism of an amino acid, such as TP, ultimately resulting in an alteration in the metabolism of a neurotransmitter substance i.e., 5-HT.

The similar changes in brain TP and possibly 5-HT metabolism present in the hypophysectomized rat and in the dwarf mouse, in which there is a more selective lack of growth hormone, point to a deficiency of the latter as a major determinant of the observed alterations. This view is supported by the finding that in both experimental models GH treatment partially counteracted the increased TP and 5-HIAA brain concentrations. The possible mode of action of GH in affecting brain TP metabolism is currently under investigation.

In conclusion, the possibility that fluctuations in some essential plasma constituents as a result of hormonal imputs may influence brain TP availability, thus altering brain 5-HT metabolism is supported by the present findings. Disease states of the endocrine system, in which dramatic changes occur in the levels of hormones in the blood may result in alteration in a brain neurotransmitter (5-HT) function. In some endocrinopaties deranged mechanisms of hormonal feed-back control and/or disorders of the affective behavior might have such an origin[17].

Riassunto. Nel topo geneticamente nano, che presenta una selettiva carenza di ormone somatotropo (GH) e nel ratto ipofisectomizzato, i livelli cerebrali di l-triptofano (TP) e di acido 5-idrossi-indolacetico (5-HIAA) sono

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considerevolmente più elevati che nei controlli, mentre i livelli cerebrali di serotonina (5-HT) sono praticamente immodificati. La somministrazione di GH riduce i livelli di TP e 5-HIAA e non modifica i livelli di 5-HT sia nel topo nano che nel ratto ipofisectomizzato. Questi risultati dimostrano che la carenza di GH è un fattore importante nel determinare le alterazioni del metabolismo della 5-HT presenti in entrambi i modelli sperimentali.

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PRO LABORATORIO

An Instrument for Mechanical Dissociation of Tissues

Mechanical dissociation of tissues into single cells results to some extent in physical damage to cells. The aim of mechanical segregation is to obtain single cells without effects of chemical dissociating agents on cellular activity; in addition it serves to free certain tissue elements prior to further treatments. An appropriate dissociation technique is expected to yield quickly and conveniently large quantities of cells, most of which are undamaged and viable. Techniques commonly used for these purposes do not completely meet all of these requirements. Tissue mincing with scissors or razor blade, or squashing tissue fragments through a net, results only in a low rate of undamaged cells; dissociation of tissues with dissecting needles does not yield sufficient quantities of isolated cells within an adequately short period of time.

The difficulty is even more severe concerning separation of the covering layer of villi, as is the case with placental villous syncytiotrophoblast. There, the syncytial nature of the layer, its size and structure makes the task of obtaining considerable amount of whole undamaged cells very difficult.

In order to overcome these difficulties, special combs were devised for mechanical dissociation of tissues. A pair of these combs were used simultaneously in the process. The dissociation was performed by movement of one comb against the other with teeth interlocking, with the fragments of tissue lying between the two rows of teeth (Figure 1). The movement of the combs resulted in dissociation of the tissue pieces into individual cells or into very small tissue fragments, according to the type of tissue.

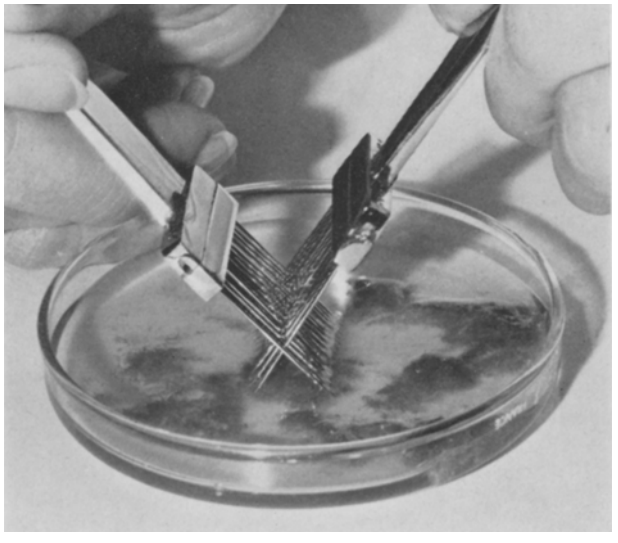

Fig. 1. The combs as used in the process of tissue dissociation.

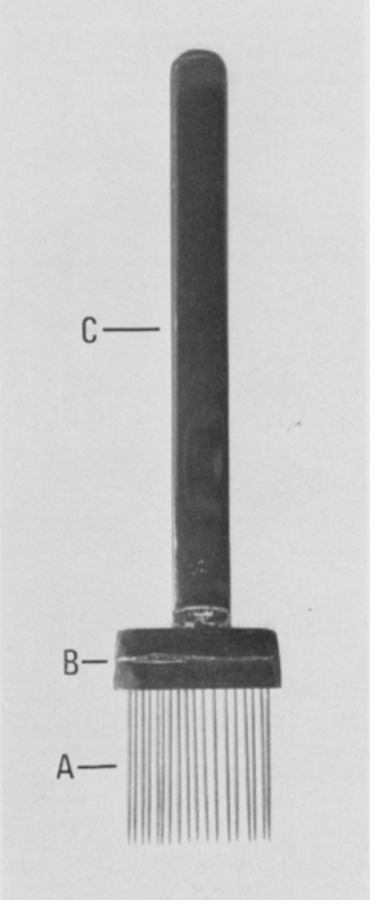

Fig. 2. A comb for tissue dissociation. A, needles; B, head; C, handle.