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CONTROL OF ADRENOCORTICOTROPHIC FUNCTION BY DIRECT INFLUENCE OF PITUITARY SUBSTANCE ON THE HYPOTHALAMUS

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The possibility of a direct action upon the hypothalamus of some substance produced by the anterior pituitary has emerged from two observations made in this department. TÖRÖK [15] investigating hypophyseal blood circulation in the living dog has noticed that in a few smaller veins of the posterior surface of the anterior pituitary blood-flow is directed upwards, *i. e.* from the hypophysis to the median eminence. The same author described a direct connection of the "capillary-loops" of the median eminence and proximal part of the stalk with the capillary net surrounding the infundibular recess of the 3rd ventricle. Erroneously the blood was considered to flow in these connections from the hypothalamus to the capillary loops. Later investigations [10] however furnished evidence for the reverse direction, *i. e.* from the capillary loops to the capillary network of the infundibular recess. — SZENTÁGOTHAI and SZÉKELY [11] observed in lizzards that the known Bodian-positive argentaffine substance of the anterior lobe is secreted from the pars tuberalis into the median eminence, where it is absorbed. — Thus the possibility of drainage of at least a small fraction of the blood from the anterior pituitary over the capillary net of the hypothalamus is given, as also the incorporation of a substance elaborated by the anterior lobe into the median eminence is indicated. Since there must be some relation between the secretion of the Bodian-positive argentaffine material by the anterior pituitary and adrenocorticotrophic activity [8, 12, 14], the idea is not far-fetched that the incorporation of anterior pituitary material into the median eminence may serve the control of adrenocorticotrophic function.

To test this hypothesis, small pieces of anterior pituitary were homografted into the hypothalamus of albino rats by the stereotaxic technique, a method successfully employed by FLERKÓ and SZENTÁGOTHAI [1] with ovary tissue. If the above-mentioned idea is correct, there must occur a depression of adrenocorticotrophic activity of the host's pituitary.

Materials and Methods

The experiments were carried out on 82 adult male wistar rats of our own breeding, kept under strictly uniform environmental conditions and on a standard diet. They were divided into 7 groups.

A) Untreated controls, 8 animals.
B) Autografts of small pieces of adrenal cortex into the hypothalamus, 10 animals.
C) Autografts of small pieces of muscle tissue into the hypothalamus, 9 animals.
D) Homografts of anterior pituitary tissue into the hypothalamus, 35 animals[1],
E) Homografts of anterior pituitary tissue into the brain (thalamus, subcortical areas), 6 animals.
F) Homografts of anterior pituitary tissue into the spleen, 6 animals.
G) Homografts of anterior pituitary tissue under the renal capsule, 8 animals.

The technique of implantation into the hypothalamus and other parts of the brain was made as described by FLERKÓ and SZENTÁGOTHAI [1]. Generally two implants were made in one animal, the amount of implanted tissue was less than 1 cu. mm. After transplantation the animals and the untreated controls were kept for 25 days under strictly uniform environmental conditions, especially at a temperature of 27 $\pm$ 1° C. After the animals were sacrificed, the weight of the hypophysis, adrenals, thyroids and gonads was determined on a torsion balance and these organs and the brain were fixed in formalin. The localisation of the hypothalamic grafts was studied on serial sections of the diencephalon, stained alternatively with haematoxylin-eosin and the MAN method, in order to differentiate also the several cell-types of the hypophyseal grafts. The hypophysis, thyroids and gonads were investigated histologically according to routine methods. One adrenal was embedded into celloidin-paraffin and sections 6 μ thick were stained with haematoxylin-eosin for the determination of nuclear size spectra, the other was cut into frozen sections for the investigation of lipids in polarized light or after staining with Sudanblack.

The nuclear size spectra were determined in the zona fasciculata according to the method of JACOBJ [7]. The diameter of 200 nuclei was measured in each case from different parts of the zona fasciculata. The nuclei being spheroid it generally suffices to measure one diameter, but if a slight irregularity was noticed the mean value of the longest and the smallest diameter was determined. To secure equal conditions during technical procedures all material was always treated together in the same chemicals.

Recently it has been reported that nuclear size in the zona fasciculata can be considered as a very good measure of adrenocorticotrophic activity [2, 4, 13, 5, 6]. Swelling of the nuclei occurs with enhanced adrenocorticotrophic activity, *e. g.* in all cases of stress or ACTH treatment, whereas a shrinkage of nuclei is found after hypophysectomy or cortisone treatment, *i. e.* when ACTH secretion is inhibited. The nuclear size is a more sensitive test than adrenal weight, since 0.1 mg ACTH/100 g body weight/day brings about a significant increase of nuclear size in 3 days. The nuclear swelling in response to increased ACTH activity (in the physiological dose range) develops after a stage of initial swelling (24 hrs; [1st phase]), then shrinking (48 hrs; [2nd phase]) and the final swelling (from the 3rd day; [3rd phase]) reaching its equilibrium on the 5th day, which is maintained in case of daily administration of the same dose (0.1 mg) for long periods [6]. The nuclear-size-test is therefore a valuable means for evaluating the level of adrenocorticotrophic activity in experiments of longer (1—4 weeks) duration. — Since nuclear swelling or shrinkage has in this case nothing to do with the so called "rhythmic" growth of nuclear volume, we do not convert the diameter into nuclear volume and in the diagram of nuclear size spectra the diameters are simply plotted on the abscissa in linear order.

Results

The weight of adrenals, thyroids and testes showed no appreciable change in groups A (controls), B (autografts of adrenal tissue), C (autografts of muscular tissue), D (homografts of anterior pituitary tissue into the hypothala-

[1] In 12 animals of this group the graft was implanted into the basal region of the anterior hypothalamus, in order to secure a connection of their blood supply with the capillary loops of the median eminence (Subgroup D_3).

mus), E (homografts of anterior pituitary tissue into non-hypothalamic parts of the brain), F and G (homografts of pituitary tissue into the spleen and under the renal capsule). The weight of the hypophysis as seen from Table 1 was practically uniform in groups A, B, C, F and G, but significantly reduced in groups D and E. No significant change was noticed in the qualitative histological picture of the adrenals in any of the groups, only in some cases of group D was there a marked reduction of birefringent lipids in the zona fasciculata. Thyroids and testes appeared histologically normal in all groups. No significant difference in histological structure was observed in the adenohypophysis.

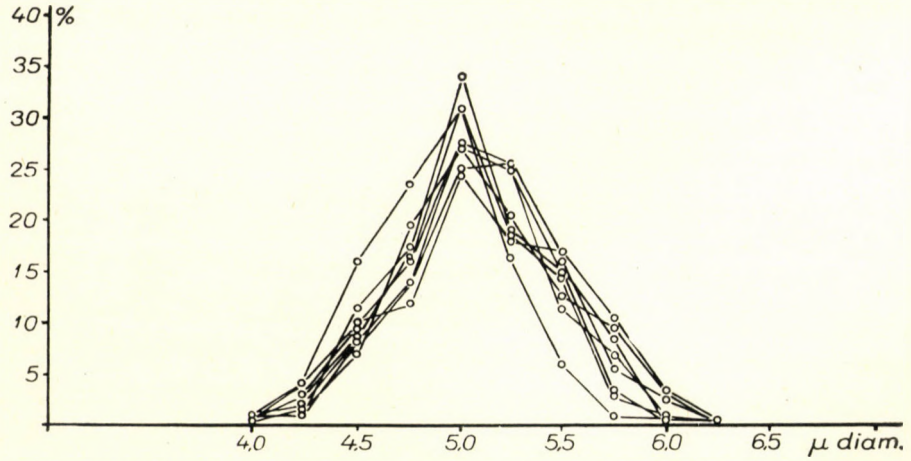

Fig. 1. Nuclear size spectra of the zona fasciculata of 8 normal male albino rats. The nuclear diameter is plotted on the abscissa, the percentual frequency of diameter classes on the ordinate

Fig. 1 shows the fairly uniform nuclear size spectra in the zona fasci culata of the adrenal cortex of normal controls (group A) with the empirical maximum in the 5 μ group.

After implantation of adrenal (group B) or muscular tissue (group C) into the hypothalamus the nuclear size in the zona fasciculata was increased in all cases without exception (Fig. 2). (A diagram showing the size spectra of group C has been omitted, being exactly the same as in Fig. 2.)

After implantation of anterior pituitary tissue into the hypothalamus (group D) the change at first appeared irregular since in about half of the cases the nuclei were enlarged and in the other half they were shrunken. After the cases had been grouped according to localization and histological character of the graft it became clear that in all cases when a graft with preserved histological structure was situated in the infundibular recess of the 3rd ventricle (Fig. 3a) the nuclear size decreased (Subgroup $D_1 = 11$

Table I

Hypophyseal weights of male albino rats of 200 ± 10 *g body weight*

Exp. group	Implanted tissue and localization of the grafts	Number of animals	Mean weight of hypophysis		
A	Normal	8	7.9 ± 0.3*		
B	Autografts of adrenal tissue in the hypothalamus	10	7.8 ± 0.1	t = 5.62 p < 0.01	
C	Autografts of muscle tissue in the hypothalamus	9	8.2 ± 0.3		t = 3.06 p < 0.01
D	Homografts of anterior pituitary tissue in the hypothalamus	35	6.0 ± 0.1		
E	Homografts of anterior pituitary tissue in non-hypothalamic parts of the brain	6	6.0 ± 0.4		
F	Homografts of anterior pituitary tissue in the spleen	6	8.1 ± 0.2		
G	Homografts of anterior pituitary tissue under the renal capsule	8	8.1 ± 0.3		

* Standard error

animals, Fig. 4) and whenever the graft was situated in the hypothalamus outside the third ventricle (Fig 3b) or had lost its histological character the nuclei were mostly enlarged (subgroup $D_2 = 12$ animals, Fig. 5). The only case in this group with shrunken nuclei was one in which the graft was situ-

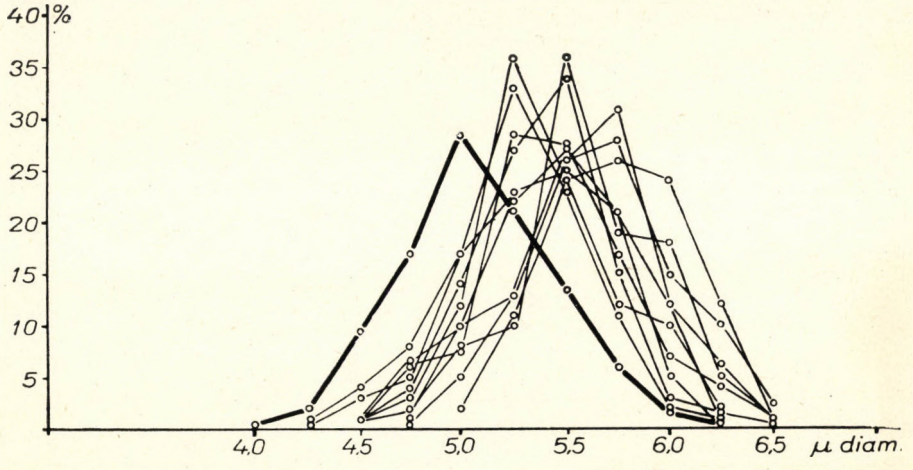

Fig. 2. Nuclear size spectra of the zona fasciculata of 10 male albino rats bearing grafts of adrenal tissue in the hypothalamus. Heavy line = average nuclear size distribution in normal group

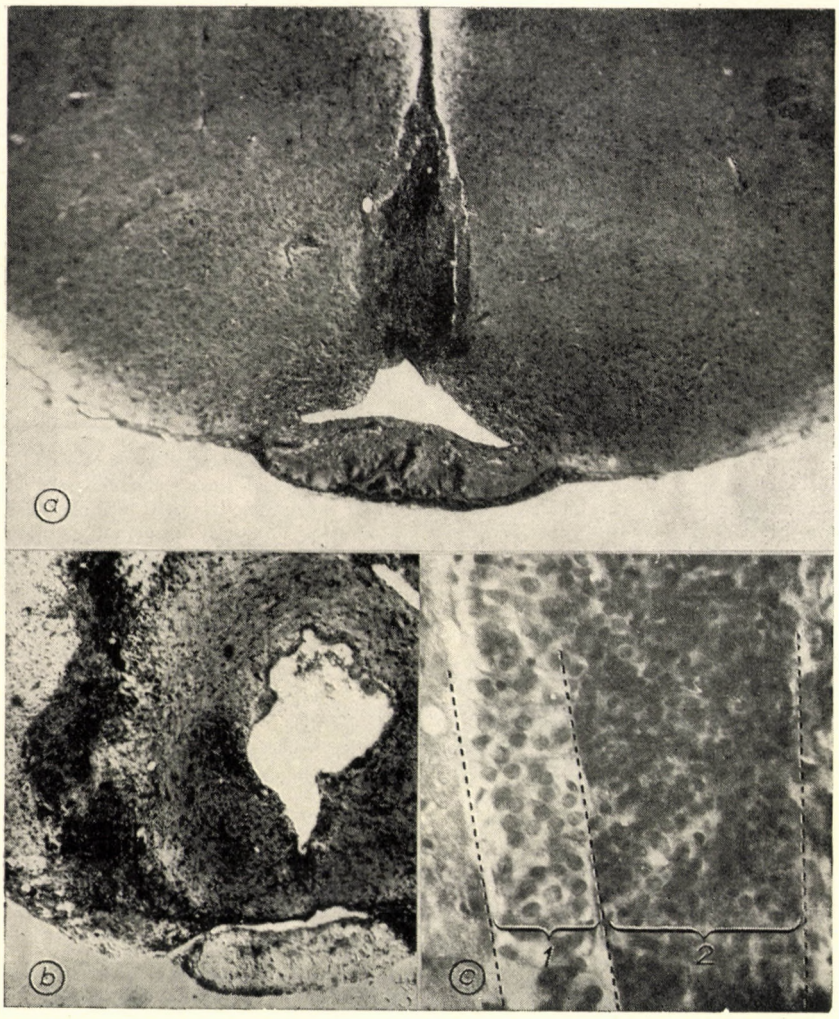

Fig. 3. a) Hypophyseal homograft in the 3rd ventricle protruding into the infundibular recess *b)* Hypophyseal homograft in premamillary region of the hypothalamus, outside the 3rd ventricle. *c)* Hypophyseal homograft in 3rd ventricle with larger magnification 1 = hyperplastic ependymal layer, 2 = grafted anterior pituitary tissue

ated in the infundibular recess but had not vascularized and was in a state of decomposition. Whenever the graft was localized in the anterior hypothalamus, and generally some part of it protruded into the 3rd ventricle but not into its infundibular recess (subgroup D_3 = 12 animals), the nuclear size was normal or somewhat decreased. Most of the hypophyseal grafts (Fig. 3c) were richly vascularized and their basophilic and acidophilic cells well preserved.

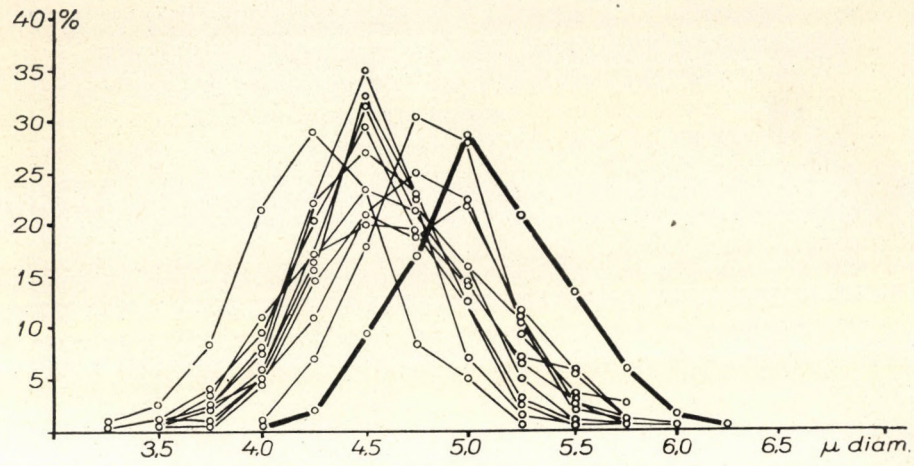

Fig. 4. Nuclear size spectra of the zona fasciculata of 11 male albino rats bearing well preserved hypophyseal homografts in the 3rd ventricle, protruding into the infundibular recess (see Fig. 3a). Heavy line = average size distribution of nuclei in normal group

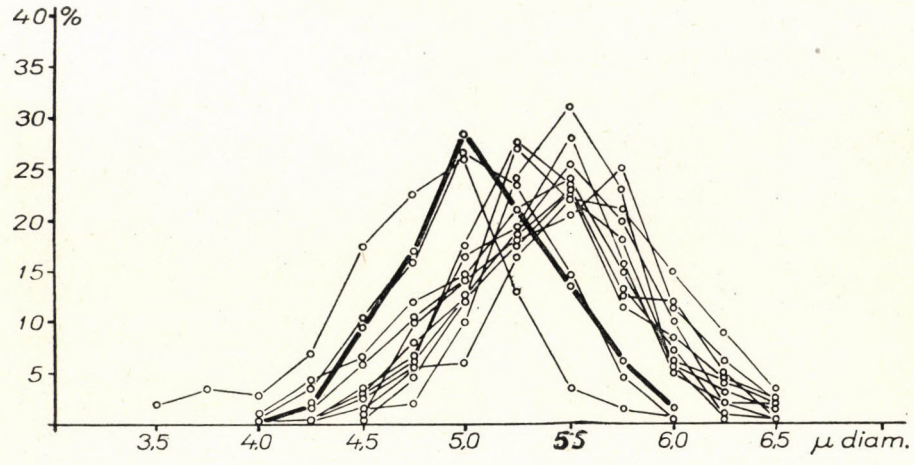

Fig. 5. Nuclear size spectra of the zona fasciculata of 12 male albino rats with hypophyseal homografts of well preserved histological structure in the hypothalamus, but outside the 3rd ventricle, or with necrosed or atrophied homografts in the 3rd ventricle. Heavy line = average nuclear size distribution in normal group

In group E with anterior pituitary homografts in non-hypothalamic parts of the brain the nuclei of the zona fasciculata were mostly but irregularly increased (Fig. 6).

From Fig. 7, where the assembled "nuclear-size-spectra" of the different groups are compared, it appears that the nuclear size was uniformly enlarged in groups B, C, E and subgroup D_2, when the hypophyseal homograft was

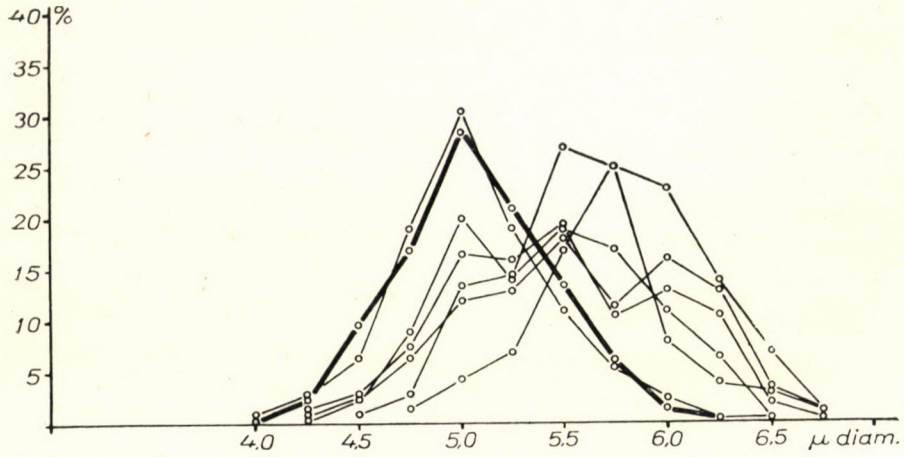

Fig. 6. Nuclear size spectra of 6 male albino rats bearing hypophyseal homografts in non-hypothalamic parts of the brain. Heavy line = average nuclear size distribution in normal group

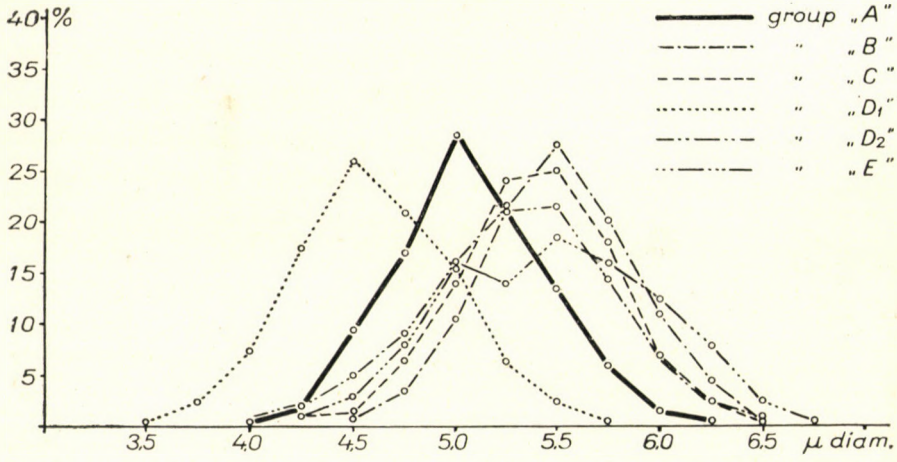

Fig. 7. Average nuclear spectra of the zona fasciculata in the experimental groups. Heavy line = average nuclear size distribution in normal group. The nuclear size is enlarged in all groups excepting subgroup D_1 (with hypophyseal homografts in the infundibular recess of the 3rd ventricle), in which nuclear size is clearly reduced

not in the infundibular recess, and decreased in subgroup D_1 with hypophyseal grafts of preserved histological structure in the infundibular recess. Statistical evaluation revealed the change of nuclear size to be significant in all groups, against group A being $t = 8.400$, $p < 0.01$ in group B; $t = 16.912$, $p < 0.01$ in group C; $t = 8{,}707$, $p < 0{,}01$ in subgroup D_1; $t = 2.976$, $p < 0{,}01$ in subgroup D_2 and $t = 4.710$, $p < 0.01$ in group E.

Discussion

As reported first by FÜLÖP [2], experimental lesions of the hypothalamus and the midbrain produce a marked increase of nuclear size in the zona fasciculata of the adrenal cortex. This enlargement of the nuclei lasts for about 3 months. The changes observed in our groups B, C and E therefore can be considered as non-specific effects of brain lesions. As mentioned in the description of the methods, nuclear enlargement of the same character is brought about by chronic administration of small doses [0.1 mg/100 g body weight/day] of ACTH. We must therefore infer that especially with hypothalamic lesions, but also with lesions in several other parts of the brain, there must occur an elevation of adrenocorticotrophic activity. — With respect to the influence exerted by adrenal corticoids on the hypothalamus, the results of group B are of some interest, — showing that adrenocortical hormones liberated directly in the tuberal region of the hypothalamus cannot depress the adrenocorticotrophic activity elevated by the lesion effect of the intrahypothalamic transplantation, as seen from the same effect produced by implantation of indifferent tissue (group C). It is of course questionable whether the adrenal implants with often not too well preserved histological structure are secreting sufficient amounts of hormone to produce any appreciable effect. But the finding is well in accord with the observation that adrenal weight [9] and nuclear size in the zona fasciculata [5] are equally depressed by cortisone administration in intact animals and animals with hypothalamic lesions.

Shrinkage of the nuclei is a much more specific effect. It is experienced exclusively after hypophysectomy, interruption of the hypophyseal stalk, or cortisone treatment. This effect can therefore be considered as a very reliable sign of depressed adrenocorticotrophic function in experiments of longer duration. This depression is the more significant, since the lesion effect of intrahypothalamic transplantation in itself enhances adrenocorticotrophic activity.

From the results of subgroup D_1 it appears that richly vascularized anterior pituitary implants with well preserved histological structure localized in the infundibular recess of the 3rd ventricle always induce this depression of adrenocorticotrophic activity, while hypophyseal implants in other regions of the hypothalamus (subgroup D_2) as well as in other parts of the brain (group E) do not bring about such an effect. This makes it clear that the effect is not caused by the hypophyseal graft itself, but by a direct action of some substance liberated by the graft at a certain site of the hypothalamus.

At present two explanations could be proposed. The blood of the graft, through connections established with the capillary loops of the median eminence, might be drained towards the portal vessels of the hypophysis. If so, some hormone — probably ACTH itself — liberated by the graft could act directly on the anterior lobe of the host and might depress adrenocortico-

trophic activity. This would correspond with the results of GEMZELL and HEIJKENSKJÖLD [3] according to which in adrenalectomized rats large doses of ACTH depress the ACTH content of the hypophysis. In this case, however, it would be difficult to explain why adrenocorticotrophic activity should not soon be stabilized on the original level. Also this assumption would not be consistent with the results of subgroup D_3, gained from a series of experiments specially designed to ascertain wether pituitary grafts, being in connection with the capillary loops of the median eminence, would influence adrenocorticotrophic function. When the pituitary graft was situated in the anterior hypothalamus, especially just behind the optic chiasma, and was connected directly with the capillary loops of the median eminence, there was no clear shrinkage, or much less than in cases of typical localization of the graft in the infundibular recess (Fig. 3a) without a connection with the capillary loops.

Another and a more probable explanation would be that some substance (probably ACTH) secreted by the hypophyseal graft might exert an influence upon nervous elements situated in the close neighbourhood of the infundibular recess, which finally results in inhibition of the ACTH secretion of the host's hypophysis. This explanation would be more consistent with the two observations mentioned in the introduction, especially with the fact that small part of the venous blood of the anterior lobe is drained trough the capillary net immediately surrounding the infundibular recess of the 3rd ventricle. The findings of GEMZELL and HEIJKENSKJÖLD [3] could be explained also in this way.

Should this explanation, which naturally cannot be considered more than a working hypothesis, stand the test of further investigations, we had a most interesting example of direct automatic self-control of pituitary function by hormonal feed-back action. A fraction of the blood draining from the hypophysis would by irrigating the region surrounding the infundibular region thus influence nervous elements according to its content of ACTH or some other substance liberated from the anterior pituitary. An automatic self-control of adrenocorticotrophic functions would be achieved if an elevation of the ACTH content of the blood returning to the hypothalamus would exert an inhibitory effect upon the nervous elements responsible for the stimulation of ACTH secretion or release.

From the data of Table 1 it is obvious that homotransplantation of hypophyseal tissue into the brain has a depressant effect on hypophyseal weight. Since about one third of an anterior lobe is grafted and generally not the whole implanted tissue survives, it can be calculated from the data that by loss of hypophyseal weight the total amount of hypophyseal tissue might be restored to the normal level.

The weight loss of the hypophysis, however, is completely independent

of the localization of the graft, hypothalamic and non-hypothalamic transplants being equally effective in this respect. This effect therefore is obviously in no connection with the depressing influence of hypothalamic pituitary grafts upon adrenocorticotrophic activity. — Implants of pituitary tissue into the spleen and below the renal capsule do not bring about such an effect. No explanation of this phenomenon can be proposed at present. It might be that only intracerebral transplants of anterior pituitary tissue can sufficiently retain their function to bring about this effect. The histological character of pituitary implants in the brain seems indeed to be better preserved than in implants of other localization.

Summary

Homotransplantation of small pieces of anterior pituitary tissue into the infundibular recess of the 3rd ventricle leads to a significant decrease of nuclear size in the zona fasciculata of the adrenals, indicative of depressed adrenocorticotrophic function. Transplantation of other tissues into this region, or homotransplantation of anterior pituitary tissue into other parts of the hypothalamus, induces nuclear enlargement in the adrenal cortex, a sign of elevated adrenocorticotrophic activity. The possibility of an automatic self-control of adrenocorticotrophic function by a direct hormonal feed-back action from the pituitary on the hypothalamus is discussed on the basis of the results.

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НЕПОСРЕДСТВЕННОЕ ОБРАТНОЕ ДЕЙСТВИЕ ПРОИЗВОДИМОГО ПЕРЕДНЕЙ ДОЛЕЙ ГИПОФИЗА ВЕЩЕСТВА НА ГИПОТАЛАМУС, И ПРОИЗВОДСТВО АДРЕНОКОРТИКОТРОПНОГО ГОРМОНА

Б. ХАЛАС и Й. СЕНТАГОТАИ

После гомотрансплантации небольшого куска ткани передней доли гипофиза в углубление воронки на дне III. мозгового желудочка величина клеточных ядер волокнистой зоны коры надпочечников сигнификантно снижается, что указывает на уменьшенную активность АКТГ. После имплантации другой ткани в данную область, или имплантации ткани передней доли гипофиза в другую область гипоталамуса, величина клеточных ядер коры надпочечников нарастает, что является признаком повышенной активности АКТГ. На основе результатов исследований выдвигается возможность саморегуляции производства АКТГ путем непосредственного действия гормона гипофиза на гипоталамус.

UNMITTELBARE RÜCKWIRKUNG EINES DURCH DEN HYPOPHYSENVORDER. LAPPEN SEZERNIERTEN STOFFES AUF DEN HYPOTHALAMUS UND DIE ADRENO. CORTICOTROPHE FUNKTION

B. HALÁSZ und J. SZENTÁGOTHAI

Homoiotransplantation kleiner Gewebsfragmente des Hypophysenvorderlappens in den Recessus infundibuli des III. Hirnventrikels ruft in der Zona fasciculata der Nebennierenrinde Kernschrumpfung hervor, was auf eine Herabsetzung der adrenocorticotrophen Hormonsekretion der Hypophyse hindeutet. Nach Transplantation anderer Gewebe an die gleiche Stelle, oder von Hypophysengewebe in andere Gebiete des Hypothalamus entwickelt sich in der Zona fasciculata eine Kernschwellung, was auf eine erhöhte adrenocorticotrophe Tätigkeit deutet. — Auf Grund dieser Befunde wird die Möglichkeit einer unmittelbaren Autoregulation der adrenocorticotrophen Hormonsekretion mittels humoraler Rückwirkung im Vorderlappen sezernierter Stoffe auf den Hypothalamus diskutiert.

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