

FUNCTIONAL REACTIVITY OF HOMOTRANSPLANTED RAT THYROID

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In our previous experiments [1, 2, 3] a technique has been elaborated for the homotransplantation of rat thyroid gland. The resistance of the host organism was controlled by transitory cortisone treatment, and the success of the transplantations was attributed to the tolerance thus induced [4]. It follows from the practical object of transplantation experiments that we must not content ourselves with a simple "take" of the transplants, with the mere survival of its cells. What we have to achieve is an organic incorporation into the host organism of the transplant, its adequate response to the actions of the organism's various regulation mechanisms.

Certain data of this kind, obtained in the course of the said experiments, were furnished by the body-weight curves of the thyroidectomized host animals and the histologically observable functional state of the transplants.

We performed some further experiments with a view to studying the functional reactivity of the transplants by means of their I^{131} uptake [5]. It was found that the transplants needed 11 days to develop such functional changes as could be attributed to the action of endogenous TSH produced by the thyroidectomized host. Since literary data showed this time to be comparatively long, we felt induced to approach the problem from another angle as well.

The usual methods for the registration of functional changes in the thyroid gland are the following.

1. Measurement of nuclear volume. It has been proved that nuclear volume increases on the administration of TSH [11, 12] and decreases on that of potassium iodide [13].

2. Measurement of the height of epithelial cells in the follicles. It has been proved that a stimulation by TSH increases cell height [9, 8, 10, 16, 6].

3. Among other cytological methods, worthy of mention is the cyclic change of esterase activity dependent on the functional state [17], or changes in phosphatase activity which latter become less pronounced on treatment with thyroxine and more marked on treatment with TSH and thiocyanate [14].

Induced by KÖNIG's report [8], we chose the second method.

Results are detailed in Table I.

Table I

Serial number of test animal	Average cell height	Standard deviation	Group
1.	3,19±0.05	0.59	Control
2.	3,23±0.09	0.91	„
3.	3.54±0.08	0.81	„
4.	2.74±0.05	0.58	„
5.	3.26±0.08	0.81	„
6.	5.29±0.08	0.84	1-day group
7.	4.85±0.08	0.83	„ „
8.	4.7 ±0.09	0.99	„ „
9.	5.27±0.09	0.96	„ „
10.	4.61±0.08	0.89	„ „
11.	4.98±0.06	0.64	2-day group
12.	4.02±0.11	1.11	„ „
13.	7.04±0.12	1.24	„ „
14.	5.32±0.09	0.95	„ „
15.	6.86±0.12	1.26	„ „
16.	6.06±0.08	0.89	„ „
17.	5.83±0.09	0.95	4-day group
18.	5.89±0.11	1.15	„ „
19.	5.15±0.1	1.05	„ „
20.	5.44±0.14	1.47	„ „
21.	6.99±0.13	1.39	„ „
22.	8.28±0.13	1.39	6-day group
23.	6.38±0.13	1.32	8-day group
24.	8.83±0.15	1.52	„ „
25.	11.03±0.2	2.03	„ „
26.	9.96±0.2	2.03	„ „
27.	10.46±0.22	2.21	„ „
28.	11.24±0.22	2.2	10-day group
29.	9.8 ±0.18	1.8	„ „
30.	8.73±0.14	1.41	„ „
31.	10.44±0.25	2.51	10-day group
32.	8.86±0.16	1.64	„ „
33.	12.29±0.19	1.93	13-day group
34.	11.66±0.25	2.51	„ „
35.	11.28±0.2	2.09	„ „
36.	7.82±0.13	1.33	„ „
37.	9.97±0.16	1.66	„ „

Table I (continued)

Serial number of test animal	Average cell height	Standard deviation	Group
38.	9.52±0.18	1.87	„ „
39.	7.94±0.14	1.45	16-day group
40.	7.77±0.22	2.29	„ „
41.	11.12±0.17	1.74	„ „
42.	11.16±0.22	2.24	„ „
43.	11.26±0.21	2.16	„ „
44.	10.44±0.2	2.0	„ „

Method

A total of 60 female albino rats with body weights between 140 and 160 g was used in these experiments. Immediately after having transplanted homologous thyroid lobes under the skin of the back of the animals (for the technique of transplantation see (1), treatment with cortisone was begun. A total amount of 15 mg, distributed over 6 doses, was administered every other day. Each animal was kept in a separate cage for 90 days after which 48 animals were thyroidectomized in the known manner (1) and 12 animals left as controls. The 48 thyroidectomized animals were divided into 8 groups, and these groups of six were then exsanguinated in chloroform anaesthesia on the 1st, 2nd, 4th, 6th, 8th, 10th, 13th and 16th days, respectively. The implants were removed from the sacrificed animals and worked up histologically in serial sections. On the 90th day, the implants were excised also from the 12 control animals and serially sectioned in a like manner. Transplants appearing unsuccessful on gross examination (16 altogether) were discarded, which of course caused a corresponding change in the original composition of the groups.

Using an eyepiece micrometer, we measured the height of 100 follicle-epithelial cells per animal in the serial sections of the removed transplants and converted the obtained relative figures into micra. We determined the average cell height per animal, its scattering, as also the dispersion of the entire assembly of measured figures. We further ascertained the mean value of the individual average heights within each group and its scattering. This done, the significance of the group differences was determined as far as it was necessary.

Figures illustrating the average height of follicular epithelium in each particular group are shown in Table II.

Table II

Group	Average cell height	Standard deviation
Control	3.19±0.07	0.74
1-day group	4.94±0.09	0.90
2-day group	5.71±0.1	1.01
4-day group	5.87±0.11	1.13
6-day group	8.28±0.13	1.39
8-day group	9.33±0.18	1.82
10-day group	9.82±0.19	1.9
13-day group	10.42±0.19	1.9
16-day group	9.95±0.19	1.98

It can be seen that thyroidectomy is followed by a progressive growth of the follicular epithelium. Such growth is marked as early as after 24 hours. To ascertain the significance of the difference between controls and tests, the 500 data obtained for the five animals of the control group were compared with the 500 data obtained for the five animals of the 1-day group by means of Wilcoxon's method. It was found that even at a probability level of 001 there was a significant difference between the two statistical assemblies.*

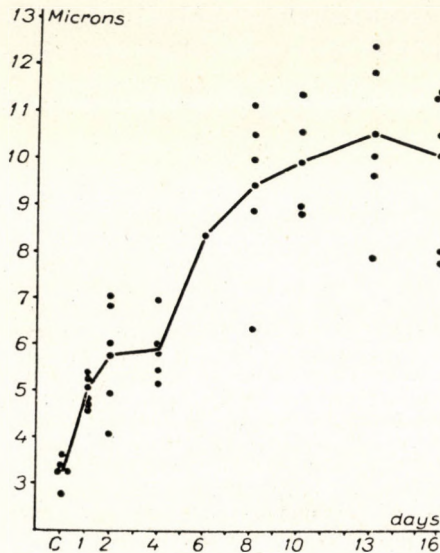


Fig. 1. Cell heights in single groups and in the animals within each group. Average group heights connected by continuous line

Figures concerning cell height in the various groups are well illustrated in Fig. 1. The dates at which the transplants were removed and analysed are shown on the abscissa, the cell heights, expressed in micra, on the ordinate. Dots mean the cell heights within each group, while the dots representing the average height of each group are connected by a continuous line.

Fig. 2 shows the percentage distribution of all cell heights in the control group, the 1-day group and the 10-day group. It can be seen that, after thyroidectomy, the curve is gradually moving to the right, becomes, at the same time, flatter and covers a gradually widening range. This is also observable from the gradually increasing dispersion values.

For the morphological illustration of the change in cell height we prepared identical enlargements of a definite visual field from various groups (Figs. 3—4). The gradual increase of cell height can be clearly followed in them,

* For the statistical analysis we are indebted to the Institute of Mathematics of the University of Debrecen.

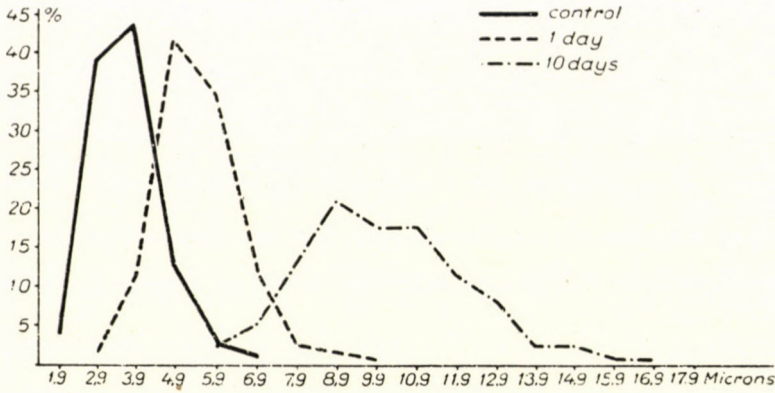


Fig. 2. Percentage distribution of cell heights in the control group, the 1-day group and the 10-day group

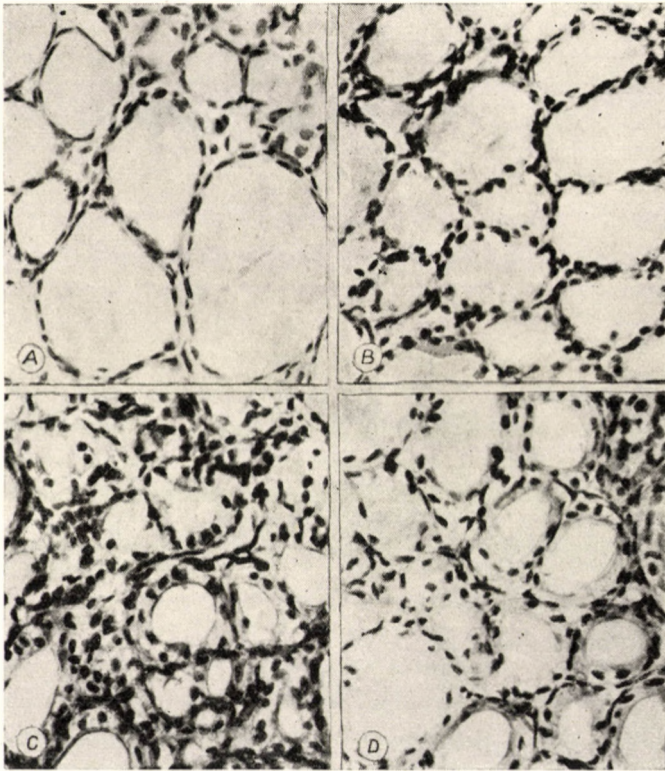


Fig. 3. 90-day transplants after treatment with adaptation doses of cortisone. HE, $\times 360$. — A: animal No. 2, control with own thyroid gland; average height of follicular epithelium, $3.23 \pm 0.09 \mu$. — B: animal No. 6. Thyroidectomized 24 hours before removing the transplant; average height of follicular epithelium, $5.29 \pm 0.08 \mu$. — C: animal No. 13. Thyroidectomized 48 hours before removing the transplant; average height of follicular epithelium, $7.04 \pm 0.12 \mu$. — D: animal No. 26. Thyroidectomized 8 days before removing the transplant; average height of follicular epithelium, $9.96 \pm 0.20 \mu$.

and they also show how the storing, inactive follicles change into more active, later almost excessively functioning, follicles.

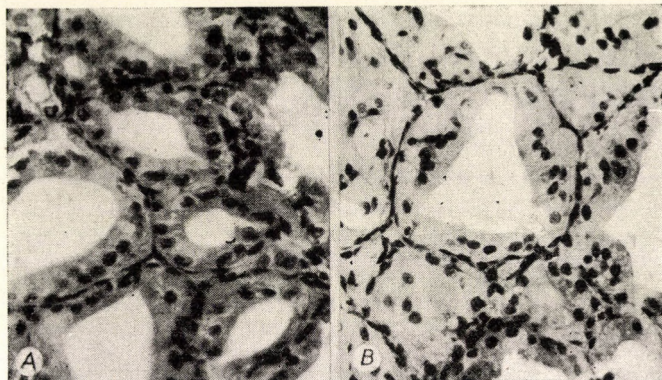


Fig. 4. 90-day transplants after treatment with adaptation doses of cortisone. HE \times 360. — A: animal No. 28. Thyroidectomized 10 days before removing the transplant; average height of follicular epithelium, $11.24 \pm 0.22 \mu$. — B: animal No. 34. Thyroidectomized 13 days before removing the transplant; average height of follicular epithelium, $11.66 \pm 0.25 \mu$

Discussion

The observation that transplants are favourably reacting to the enhanced TSH stimulus following thyroidectomy and that this response is manifest as soon as within 24 hours, seems to be the most significant result of our experiments.

A morphological change of the follicular epithelium consequent upon injection of TSH was demonstrated by JUNKMANN and SCHOELLER [7] to have occurred within 12 to 24 hours and by OKKELS [15] as early as 30 minutes after the introduction of the hormone. These authors employed other cytological methods in some of their experiments and made their observations on normal thyroids *in situ*, with arbitrary doses of thyrotropic hormone. The changes observed in our own experiments were induced by an endogenous TSH-stimulus which is undoubtedly weaker than that elicited by injected hormone; they prove that the functional value of our implants and the degree of their collaboration with the host organism were nearly as good as those of intact normal thyroids, and that, thus, implanted thyroid glands are capable of satisfactorily replacing the removed organ.

Summary

Homologous thyroid tissue was transplanted into 44 female albino rats which were treated with adaptation doses of cortisone.

The normal thyroid gland of 39 test animals was removed after 90 days and the implanted thyroid tissue was examined at various intervals following thyroidectomy. The degree

of secretory activity in the transplants was determined by measuring the height of the cells composing the follicular epithelium.

As early as one day after thyroidectomy a very significant increase in the height of the said epithelial cells could be registered. A comparison of this observation with literary data concerning the time needed by normal — *in situ* — thyroid glands to respond to stimulation by TSH permits of the conclusion that the functional value of implanted thyroids and their collaboration with the host organism are hardly inferior to those of the intact organs.

REFERENCE

1. DÉVÉNYI, I., CZENKÁR, B., ENDES, P.: (1957) Wirkung der Cortisonadaptationsbehandlung auf das Totale und erwachsene Schilddrüsen- und Nebenschilddrüsen-Homoiotransplantat der Ratte. *Frankfurt. Z. Path.* 68, 418. — 2. DÉVÉNYI, I., CZENKÁR, B., ENDES, P.: (1958) Homotransplantation of adult rat thyroid and parathyroid with simultaneous cortisone treatment. *Acta Morph. Hung.* 8, 39. — 3. DÉVÉNYI, I., CZENKÁR, B., ENDES, P.: (1958) Homotransplantation of foetal thyroid glands to temporarily cortisone-treated rats. *Acta Morph. Hung.* 8, 59. — 4. DÉVÉNYI, I., CZENKÁR, B., ENDES, P.: (1957) Untersuchung der immunbiologischen Anpassung von Homoiotransplantaten. *Frankfurt. Z. Path.* 68, 435. — 5. DÉVÉNYI, I., KERTÉSZ, L., CZENKÁR, B.: (1958) I-131 uptake of homotransplanted thyroids. *Acta. Biol. Hung.* 8, 326. — 6. GRIESBACH, W. E., PURVES, H. D.: (1943) The assay of thyrotropic activity by the cell height response in guinea-pigs. *Brit. J. exp. Path.* 24, 185. — 7. JUNKMANN, K., SCHOELLER, W.: (1932) *Klin. Wschr.* 1176. Cit.: KÖNIG (8). — 8. KÖNIG, J.: (1953) Die experimentelle Morphologie der Schilddrüse. *Med. Mschr.* 7, 624. — 9. KRACHT, J., KRACHT, U.: (1952) Zur Histopathologie und Therapie der Schreckthyreotoxikose des Wildkaninchens. *Virchow's Arch. path. Anat.* 321, 328. — 10. KRACHT, J.: (1953) Über die Regulation der Ausschüttung des thyreotropen und corticotropen Hormons. *Verh. dtsh. Ges. inn. Med.* 59. Kongress. — 11. MESS, B.: (1954) Kernvolumina der Schilddrüse als Maßstab für die thyreotrophe Aktivität des Hypophysenvorderlappens. *Acta Morph. Hung.* 4, 515. — 12. MESS, B.: (1956) Ein Verfahren zur Bestimmung kleinerer Mengen thyreotropen Hormons. *Acta Physiol. Hung.* 9, 215. — 13. MOESCH, H. R.: (1956) Zur Jodwirkung auf die Schilddrüse. *Histologische und kernvolumetrische Untersuchungen an Schilddrüsen normaler, hypophysektomierter, sowie mit Thiouracil behandelter Albinoratten.* *Acta Anat.* 27, 193. — 14. NEGRI, L., WEBER, G.: (1953) L'attività fosfatasica nella tiroide di ratto nella norma e in diverse situazioni funzionali sperimentali (trattamento con metiltiouracile, tireotropina ipofisaria, tiroxina). *Arch. "De Vecchi" Anat. patol.* 20, 253. — 15. OKKELS, H.: (1932) *Acta path. microbiol. scand.* 9, 1. Cit.: KÖNIG (8). — 16. RAWSON, R. W., STARR, P.: (1938) *Arch. int. Med.* 61, 726. Cit.: KÖNIG (8). — 17. WEBER, G.: (1954) L'attività esterastica nella tiroide di ratto nella norma e in diverse situazioni funzionali sperimentali (trattamento con tireotropina ipofisaria e tiroxina). *Arch. "De Vecchi" Anat. patol.* 22, 929.

ИССЛЕДОВАНИЕ ФУНКЦИОНАЛЬНОЙ СПОСОБНОСТИ К РЕАКЦИИ ГОМОТРАНСПЛАНТАТОВ ЩИТОВИДНОЙ ЖЕЛЕЗЫ КРЫС

И. ДЕВЕНЬИ и Л. МЕДЬЕРИ

Авторы проводили гомотрансплантации щитовидной железы крыс на 44 самках белых крыс, применяя одновременно обработку животных адаптационными дозами кортизона, согласно разработанной ими методики. Они удалили собственные щитовидные железы у животных-хозяев 90-дневных трансплантатов, а затем исследовали в различных периодах трансплантаты и регистрировали степень секреторной активности последних путем измерения высоты фолликулярных эпителиальных клеток.

Уже за день после удаления щитовидной железы наблюдалось сильно сигнификантное повышение высоты фолликулярных эпителиальных клеток. Сопоставляя полученные результаты с литературными данными о времени реакции нормальных щитовидных желез на стимул TSH, указывается на то, что функциональная ценность трансплантатов, их взаимодействие с организмом животных-хозяев сильно подходит к функциональной ценности нормальной щитовидной железы.

UNTERSUCHUNG DER FUNKTIONALEN REAKTIONSFÄHIGKEIT VON HOMO-
TRANSPLANTIERTEN RATTENSCHILDDRÜSEN

I. DÉVÉNYI und L. MEGYERI

An 44 weissen weiblichen Ratten wurde Homotransplantation von Rattenschilddrüse durchgeführt, bei gleichzeitiger Behandlung der Tiere mit von den Verfassern ausgearbeiteten Kortisonadaptationsdosen. 90 Tage nach der Transplantation wurde die eigene Schilddrüse der Akzeptortiere entfernt, und danach die Transplantate in verschiedenen Zeiträumen untersucht. Der Grad der sekretorischen Aktivität der Transplantate wurde mittels Messung der Höhe der Follikel epithelzellen registriert.

Bereits nach Verlauf eines Tages nach der Ektomie konnte eine stark signifikante Zunahme der Höhe der Follikel epithelzellen beobachtet werden. Ein Vergleich dieses Ergebnisses mit den literarischen Angaben über die Reaktionszeit von normalen Schilddrüsen auf TSH Stimulation weist darauf hin, dass der funktionelle Wert der Transplantate, ihr Zusammenwirken mit dem Wirtsorganismus, dem Wert der normalen Schilddrüsen stark nahekommt.

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