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HOMOTRANSPLANTATION OF ADULT RAT THYROID AND PARATHYROID WITH SIMULTANEOUS CORTISONE TREATMENT

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In an earlier communication [9] we reported on the results of investigations concerning homotransplantation of embryonic rat thyroid to thyroidectomized hosts treated with cortisone. In the present paper experiments will be described in which the thyroid gland of adult rats was homotransplanted.

Method

Sexually mature white rats of both sexes derived from an impure strain were used in the present experiments. In exstirpating the thyroid glands we adopted the method described by GRIESBACH and PURVES [15]; the operation was performed with all surgically possible radicality which really means subtotal resection. The parathyroids were removed together with the thyroid lobes.

Transplantation took place 1 to 3 days after thyroidectomy. Under ether anaesthesia we removed the larynx *in toto*, detached the thyroid gland and parathyroid after which the animals were killed. Using one lobe per host, the transplantation, performed without delay, was carried out by transferring the transplant into a subcutaneous pocket behind the right scapula through a small aperture made on the shaven back of the host animal. The incision was closed with clips which were removed on the seventh day.

Following transplantation, 2,5 mg of cortisone (Cortone Acetate, Merck.) was injected subcutaneously every second day six times in succession, thus making a total of 15 mg distributed over 11 days.

We weighed the animals every week.

A total of 38 animals was used in the following experimental groups (Table I).

Table I

Experimental groups

Group	No. of animals	Thyroidectomy	Cortisone treatment	Time of histol. examination at	No. of successful transplantations
I.	9	+	∅	5 to 60 days	∅
II.	10	∅	+	30 to 230 days	7
III.	19	+	+	3 to 300 days	16

Animals to be histologically examined were bled in chloroform anaesthesia. Transplant and remnant of own thyroid, together with larynx, were fixed in a 10 per cent solution of neutral formalin, then imbedded, serially sectioned and stained with haematoxylin-eosin.

Results

Group I (Controls). The transplants, seated in the loose subcutaneous connective tissue and surrounded by a thin connective-tissue capsule, had a size of 3 to 4 × 2 to 3 × 2 mm, and their colour was first greyish red, later greyish white. All transplants of this group went through the various phases of destroying process well-known in homotransplantations. There is no need to describe the process in detail as it has already been treated by a number of authors [8, 27, 28]. After the 30th day not even remnants of the thyroid and parathyroid tissues were observable ; their place was occupied by increasingly fibrous granulation tissue. Within the 60 days of observation there was just a slight loss of body weight. Only 2 animals exhibited tetanic spasms and spasmophilia between the 7th and 14th day : these symptoms were but transitory and subsided during the further course of observation.

After the 40th day regenerative hyperplastic nodes with functioning follicles were encountered in the residual thyroid.

Group II. The results obtained from the 10 analyzed transplants of this group are shown in Table II.

Table II

Results in group II

Age of transplant days	Condition of transplanted thyroid and parathyroid
30	regressing
70	successful
90	,,
110	,,
130	,,
150	,,
170	unsuccessful
190	successful
210	,,
230	unsuccessful

Table II shows that the transplants examined on the 30th, 170th and 230th day, respectively, had perished or were perishing. The picture of the 30-day transplant was similar to that of the same age in Group I. There was extensive central necrosis, largely replaced by granulation tissue ; the remaining marginal follicles were pushed apart by lymphocytes ; there were many lymphocytes in the surroundings.

The transplant in the 170 and 230-day old animals appeared on gross examination as a 2 to 3-mm wide greyish spot. Under the microscope complete

fibrosis with siderophage cells was revealed with no trace of thyroid or parathyroid tissue.

The 7 successful transplants appeared as pale reddish-brown figures embedded in loose subcutaneous tissue, surrounded by a thin transparent capsule ; their size was 3 to 4 × 1 to 2 mm. Situated in an uninflamed environment, there appeared actively storing follicles divided into lobules by septa of connective tissue ; they were surrounded by a thin connective-tissue capsule (Fig. 1). There were some siderophages in the septa. The epithelial lining of the follicles was flat, consisted of one layer, with colloid in the lumen. Parathyroid with normal structure was preserved in all of the 7 animals (Fig. 2).

No lymphocytic infiltration, a characteristic sign of deterioration, could be observed in these transplants. At the edge of some there were lymphocyte masses 80 to 100 μ in diameter (Fig. 1). These did not seen in any way different from the lymphatic follicles found next to the thyroid gland.

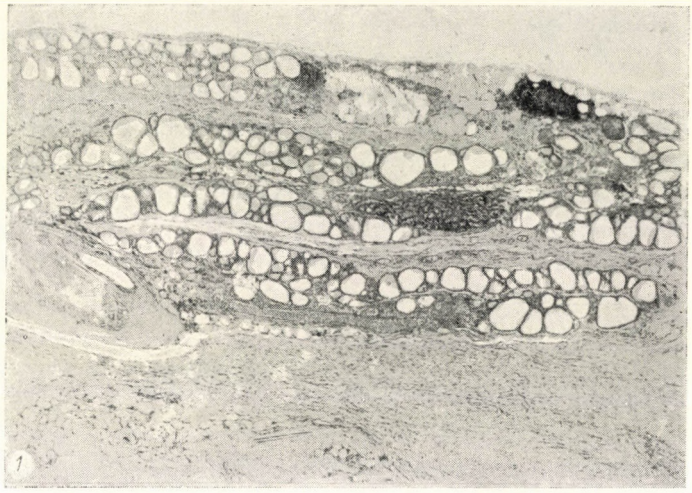
The increase in body weight was normal.

Group III. The results of the 19 transplants of this group are listed in Table III.

Table III

Results in group III

Age of transplant days	Condition of transplanted thyroid and parathyroid
3	transitory
7	,,
13	,,
17	,,
25	successful
31	,,
40	,,
50	,,
60	,,
86	unsuccessful
90	successful
98	,,
102	regressing
135	unsuccessful
140	successful
150	,,
180	,,
250	,,
300	,,



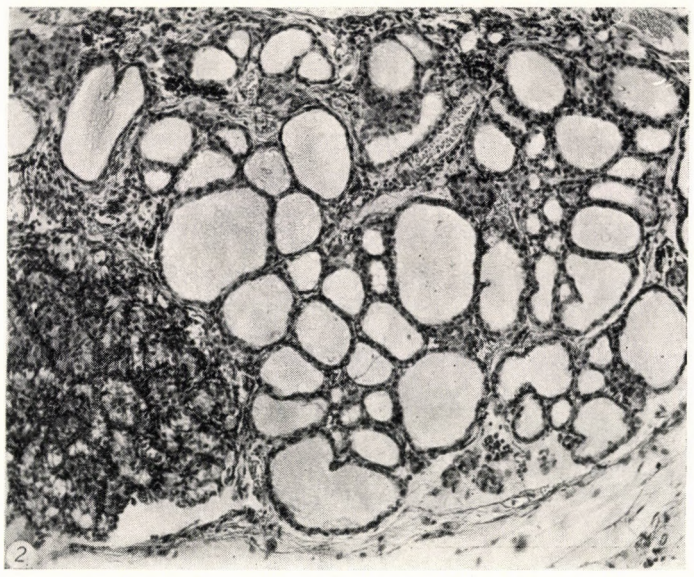


Fig. 1. 190-day old transplant. Group II. Thyroid divided into lobules by septa of connective tissue. Upper right corner : lymphocyte collections at the edge of thyroid. Haematoxylin-eosin

Fig. 2. 150-day old transplant. Group II. Uninflamed thyroid and parathyroid. Flat epithelial lining of follicles, with colloid in the lumen. State of active storage. Haematoxylin-eosin; ×130

Table III shows 16 transplantations to have been successful and 3 to have failed. In the two transplants marked as unsuccessful, i. e. the 86-day and the 133-day ones, we encountered only cicatrization with sporadic siderophage cells. The thyroid in the regressing 102-day old transplant appeared to have been destroyed by proliferating connective tissue. The scattered follicles were tiny, atrophied, with a flat epithelial lining and devoid of colloid (Fig. 3).

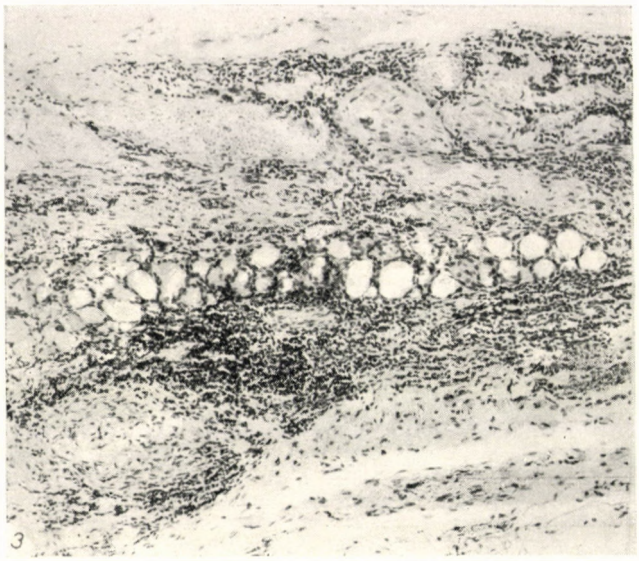


Fig. 3. 102-day old transplant. Group III. Regressing transplant, infiltrated with lymphocytes and macrophages, in a state of transformation into connective tissue. Follicles atrophied, with flat epithelial structure, devoid of colloid. Lower left corner : parathyroid in a similar condition. Haematoxylin-eosin ; × 110

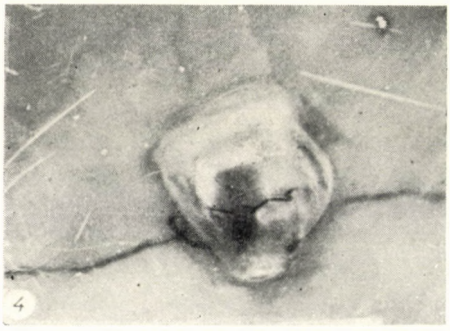
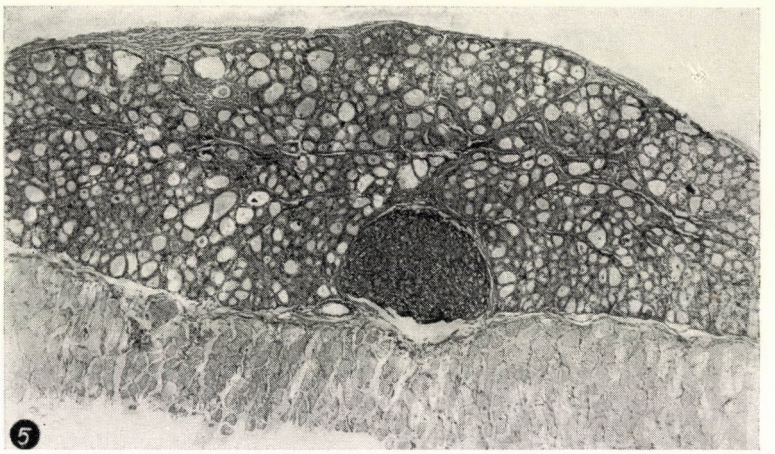


Fig. 4. 140-day old transplant. Group III. Surrounded by rich and wide-spread vascular network. Picture taken after preparation of back-skin

All of these theree animals became cachectic, two of them (the 102 and the 133-day old ones) developed pneumonia ; all died. No tetanic symptoms were observed in any of them.

The remaining 16 successful transplants measured 3 to 5 × 3 to 4 × 2 to 3 mm ; their colour was brownish-red ; they were surrounded by a very rich and wide-spread collateral vascular network (Fig. 4).

Histologically, the initial destruction of the central gland in the transplants marked "transitory" becomes less and less pronounced and, leaving



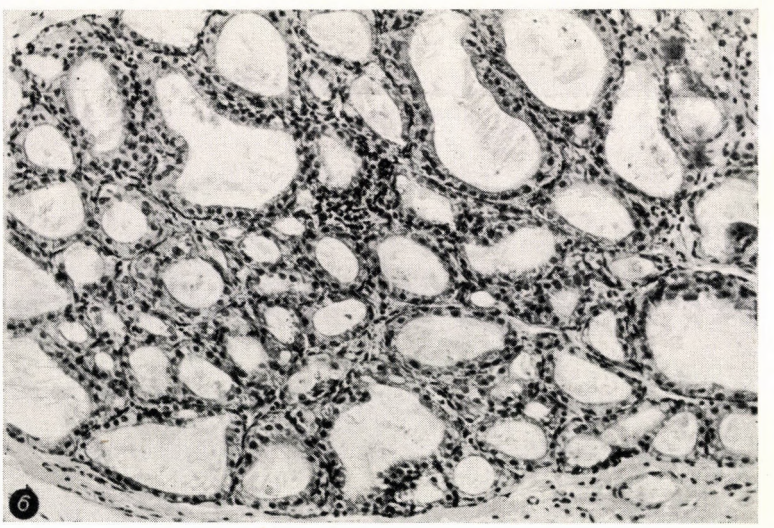


Fig. 5. 250-day old transplant. Group III. Completely intact, uninflamed thyroid and parathyroid. Lobules formed by septa of connective tissue. Haematoxylin-eosin

Fig. 6. 140-days old transplant. Group III. Part of actively-storing thyroid. High epithelial structure in follicles, with some thin colloid in the lumen. Amount of stroma corresponding to that in normal glands, richly vascularized. No inflammatory infiltration. Haematoxylin-eosin ; × 150

behind a certain amount of connective tissue, disappears later altogether. This initial, transitory, partial necrosis seems to be a phenomenon common to all transplants because nutriment has to be taken up by way of diffusion until the development of a new blood circulation during which the central parts are usually necrosed.

No lymphocytic infiltration, characteristic of destruction was encountered in the successful transplants. It was only along the capsule that collections of lymphocytes, similar to those mentioned in Group II, were observed. The transplanted thyroid displayed variable activity. The follicles were lined with high, vacuolized epithelium, with or without a little thin colloid. The thyroid gland was divided into lobules by thicker bundles of connective tissue ; the

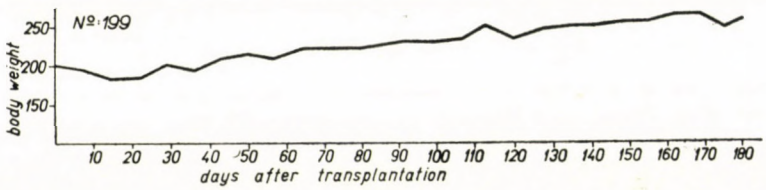


Fig. 7. 180-day transplant. Group III. Animal No. 199. Almost normal increase in body weight. Weight curve of other animals of Group III was similar

stroma within these lobules corresponded to that in normal glands and was richly vascularized, with free capillaries. The parathyroids were likewise preserved in all cases, their structure was normal (Figs. 5 and 6).

The body weight, after an initial loss due to cortisone, showed a normal or almost normal increase from the 21st day (Fig. 7). No tetanic symptoms appeared.

There was adenomatous hyperplasia in the remnant of the animals' own thyroid gland, its degree of activity keeping on a level with the transplant.

Discussion

The purpose of the present experiments was to observe the effect of cortisone treatment and thyroidectomy in animals with thyroid and parathyroid homografts.

The fact that endocrine organs, such as the thyroid gland, having no efferent ducts and being comparatively less dependent on the nervous system, are easily transferable and able to provide the whole organism with their hormones after the development of the new circulation, makes them eminently suitable for the study of transplantations [13]. If they are grafted on the appropriate site, it is always possible to tell if one has to do with a genuine "take"

or simply with the proliferation of the tissues of the host. This we deem to be very important, for many of the numerous contradictions in the literature on transplantation are largely due to obscurities in this respect. We speak of transplantations when cartilage, bone, cornea or vessels are transferred although all these tissues serve only as supporting structures for the invading tissue of the host, while they, themselves, do not survive. In order to bring out this difference, LONGMIRE [32] suggests the introduction of the terms "homovital" and "homostatic" transplantation. Homotransplantation of adult thyroid tissue has been described by numerous authors. Recent comprehensive and experimental works are unanimous about the failure of genuine homotransplantations [6, 8, 12, 24, 27, 28, 29, 30, 31, 32, 35, 44].

In our present experiments, 16 out of 19 thyroids homotransplanted to thyroidectomized and cortisone-treated rats were found to have survived even after 300 days of observation. Survival in equally treated but not thyroidectomized hosts was observed in 7 out of 10 cases, the oldest observed transplant having reached an age of 210 days. In the control group, on the other hand, i. e. in hosts thyroidectomized but not treated with cortisone, none of the 9 transplants survived the 60th day.

Against any possible and seemingly not unjustified objection, that the success of the above-described transplantations may have been due to inbreeding, we can point to the fact that, as has already been mentioned, all rats used in the present experiments came from an impure strain, so that inbreeding can have played no part in the outcome of the experiments. Besides, it is known from LOEB's investigations [30] that individual differences in rats cannot be made to disappear by inbreeding. Moreover, it was observed also by LOEB [29] that, as regards rats, it is only in the quantity of the successful cases and not in their quality that syngenesiotransplants, i. e. transfers within inbred groups, differ from true homotransplantations. To our knowledge, not even in syngenesiotransplantations have grafts been observed that would have retained their full vitality during 150 to 300 days, as did our homotransplants.

HALSTED's statement [16] that the lack of the hormone of an endocrine organ promotes the successful transfer of that same organ has been accepted as an axiom in literature. Since then, a great number of investigators have availed themselves of this principle [6, 10, 25]. Some authors modified it in the sense that it is not the lack of the hormone of a given organ but the amount of the trophic hormone which constitutes the decisive factor [41, 47].

Our investigations, in agreement with the observations made by DEMPSTER and DONIACH [8], throw an entirely different light on HALSTED's principle. The experiences made in our three experimental groups afford the following conclusions 1. The mere absence of an endocrine organ does not warrant a successful "take" of, or prevent the immune-biological reaction destructive to, the transplant. 2. If immune-biological reactions are effectively inhibited

by the administration of cortisone, the absence of the corresponding endocrine organ does not manifest itself in the success or failure of the "take" but, once the transplant has "taken", in the degree of its activity.

That this is so is shown by the collateral result of one of our experiments with parathyroids. It is known that rats have, over and above those attached to the thyroid gland, a number of accessory parathyroids in the pharyngeal wall and chiefly around the thymus [20]. We encountered them in most of our animals. Although, according to HALSTED's theory, this ought to have prevented transplantations from being successful, our homotransplanted parathyroids survived in all cases nevertheless.

Since MEDAWAR's classic experiments [34] it has been generally accepted that immune-biological factors are at play in the destruction of homotransplants. The immune-biological reaction arises from the incompatibility between transplant and host. That the destruction of transplants is of an immune-biological nature has since been confirmed by many experiments [3, 6, 7, 10, 33, 36, 39, 45, 48].

Against this, KNAKE [21, 22, 23], far from regarding the destruction of transplants as a problem of immune biology, attributes it to vascular alterations, claiming that it is inadequate vascularization to which deterioration is due. Holding the technique of transplantation to be the decisive factor, he tries to circumvent the problem of how to nourish the young transplant during the absence of vessels by transferring only small pieces of tissue. And yet, the picture with which he wants to demonstrate a successful transplant [21] appears to us to represent rather a scar tissue infiltrated with lymphocytes. *Intact tissue structure, adequate functioning, and a complete incorporation into the host organism are, in our opinion, the criteria of successful transplantation.*

The results we have obtained from the treatment with cortisone make us inclined to accept the first theory. The effect of cortisone on antibody production [6], reticuloendothelial-activity [18] and hypersensitivity [2, 17, 41, 42] is well-known. Likewise well-known is its action which causes the involution of senescent lymphatic tissues [46] and inhibits the reactivity of all elements of the connective tissue [19, 38].

Cortisone has been successfully used in a great variety of transplantation experiments [2, 5, 6, 26, 40, 42, 43] although, in skin grafts, all that has been achieved was a longer survival of the transplant.

PATTERSON et al. [37] claim that a discontinuance of the cortisone treatment kills the transplanted skin, however well it had adapted itself. Reports concerning ACTH therapy are contradictory [4, 11].

Treatment with cortisone has decidedly proved successful in our experiments. This, apart from what has been said above, may have been due to the fact also that cortisone, if introduced into organisms in a state of hyperthyroidism, increases TSH production [1]. That ACTH has a rather feeble effect

may be due to the fact that endogenous corticoid production does not even amount to the dose used in our experiments.

As far as we can judge, cortisone, by blocking the immune-biological processes of the host organism, prepares — in the majority of cases — the ground for a certain biological adaptation of the transplant so that, once the process of adaptation has been accomplished, histological incompatibility may disappear. GREENE [14] has adopted a similar view. This theory does not tally with the observations of PATTERSON et al. [37] who observed heterotransplanted tumours to perish after the discontinuance of cortisone treatment.

What we saw in our own experiments was that a comparatively short (11 days) initial treatment with a total dose of 15 mg of cortisone sufficed to keep the transplants alive. Investigations concerning the mechanism through cortisone acts, and the conditions of the above-mentioned adaptation, are now being conducted.

One of the chief problems on which these investigations are expected to throw light is the said biological aspect of the transplantations, while — on the other hand — the question of a practical application of the results so-far achieved seems already justified. Nowhere in the literature have we encountered reports on the homotransplantation of thyroids performed under easily practicable conditions that would have given results comparable with those achieved in the present experiments.

Summary

Adult rat thyroids were homotransplanted to intact and thyroidectomized rats, subjecting the hosts at the same time to a transitory treatment with cortisone.

Out of 10 thyroid glands, homotransplanted to rats which had unimpaired thyroids and were given a total dose of 15 mg of cortisone distributed over 11 days, 7 were found to have survived during a time of observation which lasted 30 days in the shortest and 230 days in the longest instance.

Out of 19 thyroid glands, homotransplanted to thyroidectomized rats which received a similar treatment with cortisone, 16 were found to have survived during a time of observation which lasted 3 days in the shortest and 300 days in the longest instance.

The results of the present experiments give a new interpretation to HALSTED's theory.

Intact tissue structure, adequate functioning, and a complete incorporation into the host organism are thought to constitute the criteria of successful transplantation.

It is suggested that an initial treatment with cortisone, by blocking the immune reactions of the host, makes it possible for the transplant to develop a certain biological adaptation to the host organism.

The question of a practical application of the method as described in the paper is raised.

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ГОМОТРАНСПЛАНТАЦИЯ ЩИТОВИДНОЙ И ОКОЛОЩИТОВИДНОЙ ЖЕЛЕЗ ВЗРОСЛЫХ КРЫС ПРИ ЛЕЧЕНИИ КОРТИЗОНОМ

И. ДЕВЕНЬИ, Б. ЦЕНКАР и П. ЭНДЕШ

Авторы проводили гомотрансплантации щитовидной железы взрослых крыс на нормальных животных и на животных с удаленной щитовидной железой, вводя им некоторое время Кортизон.

Лоскуты щитовидной железы, гомотрансплантированные в 10 крыс с неповрежденной щитовидной железой — при даче всего 15 мг Кортизона в течение 11 дней после пересадки — давали, на основании исследований, проведенных от 30—230 дней, в 7 случаях успешные трансплантаты.

Гомотрансплантированные лоскуты щитовидной железы в крысы с удаленной щитовидной железой, при одинаковом с вышеописанным лечением Кортизоном, давали, на основании исследований, проведенных от 3—300 дней, в 16 случаях успешные трансплантаты.

На основе своих исследований авторы выдвигают новое объяснение принципа Галстеда.

По их мнению критерием успешности пересадки является неповрежденная тканевая структура, соответствующая функция и полное врастание в организм хозяина.

Авторы предполагают, что начальное лечение Кортизоном, прекращением реакции иммунизации организма хозяина, создает для трансплантата возможность известной биологической адаптации к организму хозяина.

Авторы выдвигают вопрос о возможностях практического применения данного метода.

HOMOTRANSPLANTATION VON SCHILDDRÜSEN UND NEBENSCHILDDRÜSEN ERWACHSENER RATTEN BEI CORTISONBEHANDLUNG

I. DÉVÉNYI., B. CZENKÁR und P. ENDES

Homotransplantation erwachsener Rattenschilddrüsen wurde an intakten und thyreoidektomierten Ratten bei vorübergehender Cortisonbehandlung durchgeführt.

Homotransplantation bei 10 Ratten mit intakten Schilddrüsen mit 11tägiger, nach der Transplantation ausgeführter Cortisonbehandlung mit insgesamt 15 mg Cortison ergab nach 30—230 Tagen in 7 Fällen erfolgreiche Transplantate.

Homotransplantation bei 19 thyreoidektomierten Ratten war mit gleicher Cortisonbehandlung, auf Grund von in 3—300 Tagen vorgenommenen Untersuchungen in 16 Fällen erfolgreich.

Auf Grund der Untersuchungen wird eine neue Interpretation des Halstedschen Prinzips gegeben. Das Kriterium eines erfolgreichen Transplantates wären eine intakte Gewebsstruktur, entsprechende Funktion und vollständiger Einbau in den Organismus des Wirtes.

Es wird angenommen, dass die anfängliche Cortisonbehandlung durch die Abstellung der Immunreaktionen des Wirtsorganismus für die Transplantate die Möglichkeit einer biologischen Anpassung zum Wirtsorganismus schafft.

Es werden auch die Möglichkeiten der praktischen Verwendbarkeit dieser Methode besprochen.

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