

## TRANSPLANTATION OF REGENERATIVE HEPATIC TISSUE

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In the course of transplantation experiments that have been in progress in our Department for some time it was possible to demonstrate that heterotransplants — and presumably also homologous transplants — pass through three phases in the host organism; *viz.* (i) the period of local nutritional disturbance; (ii) the period of assimilation-adaptation; and (iii) that of the development of immune materials. We attach a particular importance to the second phase during which the implanted heterologous tissue has to combat the harmful influences of and adapt itself to the new milieu, and must become capable of assimilating foreign proteins. This is the phase which we think determinates the destiny of the transplant: negatively if it perishes and positively if it survives this phase, whereafter the chances of its further existence are favourable [1, 2, 3, 7, 8].

It has been observed in the course of these experiments that, if autologous hepatic tissue had been transplanted into the anterior eye chamber of rats, the parenchymal cells degenerated, while the biliary ducts began to proliferate and formed islets of hepatic parenchyma. This was a remarkable phenomenon considering that the liver tissue of adult rats practically never survives explantation on heterologous medium and proliferates very poorly even in autologous media [2]. Only regenerative adult liver is able to thrive under such conditions. We had therefore to assume the existence of factors which activate the fragments of tissue taken from intact liver and transplanted into a regenerating organism, factors which make the vitality of the transplants similar to that of regenerative tissue.

The purpose of the experiments to be reported was to ascertain whether direct (local) or humoral factors of the regenerative process are at play at transplantation, and to control some theoretical considerations regarding transplantation of tissues of increased vitality.

### Method

A total of 64 albino rats of 200 g body weight each was used in the experiments. Each experimental group consisted of 8 animals into both eyes of which hepatic tissue was implanted. The transplantations were partly homologous and partly heterologous, with mice as donors

in the latter case. The tissue to be transplanted was cut up into fragments of 0.5 cu. mm and inserted into the lumen of an injection cannula with a blunt point. After opening the anterior chamber of the recipient's eye by means of Graefe's lancet, the cannula was introduced through the aperture and the tissue-fragment was placed into the anterior chamber, strictly observing the rules of sterility.

Likewise Graefe's lancet was used to injure the liver of the recipient animals. After opening the abdomen, a wound some mm wide and deep was inflicted on the liver, covered with sterile thrombin-fibrin powder to prevent haemorrhage, and the abdominal wall was closed. Transplantation was performed 24 hours after the operation.

The technique of injuring the liver of the donor animals was the same as in the case of the recipients; transplantation followed 48 hours later, with uninjured tissue taken from the edge of the wound.

The recipients' eyes were removed and fixed in Carnoy's fluid 10 and 20 days after the transplantation. After embedding the area of the anterior chamber in methyl-benzoate-celloidin-paraffin, serial sections were made and stained with methyl green-pyronine.

The period of observation lasted 20 days.

### Experimental

The experiments were carried out in four groups: (i) intact hepatic tissue was transplanted into rats with intact liver; (ii) intact hepatic tissue was transplanted into rats with their liver injured; (iii) hepatic tissue from animals with a damaged liver (regenerative tissue) was transplanted into rats with intact liver; (iv) regenerative hepatic tissue was transplanted into rats with impaired (regenerative) liver. All these transplantations were made homologously and heterologously alike. Since no difference was observed in the behaviour of homologous and heterologous transplants, the results will be discussed without regard to this factor.

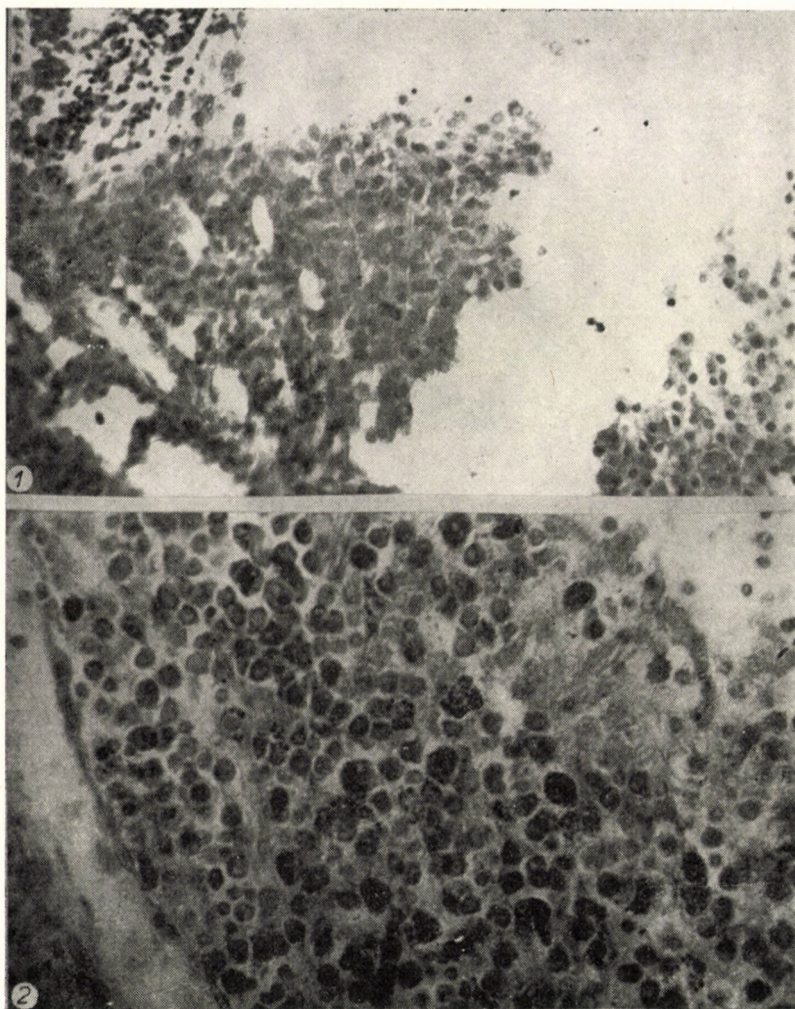
#### *(i) Transplantation of normal liver into the anterior chamber of rats with intact liver*

Ten days after the operation there was no nuclear staining observable in the area of the disintegrated graft. Pyroninophilia was slight and non-specific. Plasmocytes were accumulated in the marginal parts of the transplant, while occasional macrophages containing haemoglobin appeared nearer the centre.

Twenty days after the operation, the structure of the transplanted tissue-fragment had become pervaded by capillaries and was no longer recognizable so that only proliferating connective tissue marked the site of the vanished transplant. The plasmocyte and macrophage count had increased since the 10th postoperative day. The process of absorption was very marked.

#### *(ii) Transplantation of normal liver into the anterior chamber of rats with impaired liver*

On the tenth postoperative day the transplant was smaller than its original size, with a necrotic zone in its central part and a slight plasmocytic reaction. Islets of regular hepatic cells were found in several areas, with

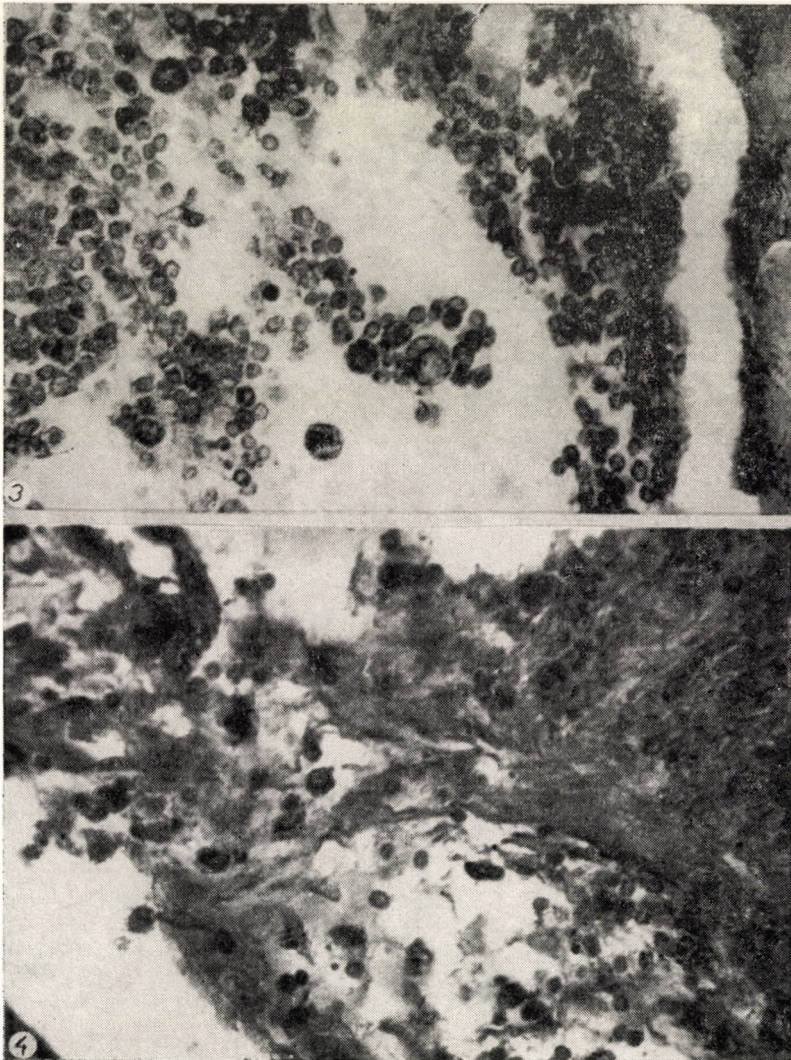


*Fig. 1.* Heterologous regenerative hepatic tissue transplanted into rat with liver injury, after 20 days. Note intact liver-cell trabeculae. Methyl green-pyronine,  $\times 100$

*Fig. 2.* Homologous regenerative hepatic tissue transplanted into rat with unimpaired liver, after 20 days. Marked degeneration, many plasmacytes and macrophages. Hepatic tissue not recognizable. Methyl green-pyronine,  $\times 200$

pronounced nuclear staining of the cells and very marked pyroninophilia of the cytoplasm. The reduction of the necrosed area was associated with a decrease in the number of macrophages.

On the twentieth postoperative day, the hepatic structure was still preserved and in some parts even more pronounced than in the 10-day preparation. Marked capillarization was seen.



*Fig. 3.* Heterologous regenerative hepatic tissue transplanted into intact animal, after 10 days. Changes as in Fig. 2. Methyl green-pyronine.  $\times 200$

*Fig. 4.* Homologous intact hepatic tissue transplanted into intact animal, after 10 days. Note almost complete absorption. Methyl green-pyronine.  $\times 200$

*(iii) Transplantation of regenerative liver to the anterior chamber of rats with intact liver*

After 10 days the general picture was as in (ii). Central necrosis, plasmocytes in the marginal parts, islets of hepatic cells, regular structures and staining were characteristic.

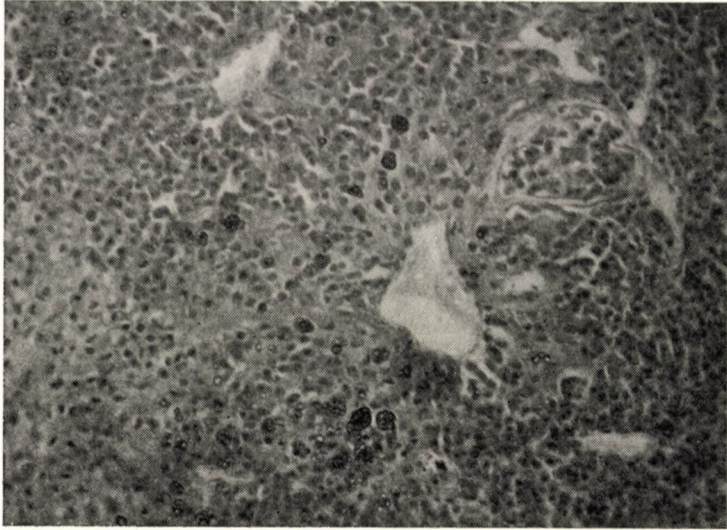


Fig. 5. Heterologous intact hepatic tissue transplanted into rat with liver injury. Note regular structure and occasional macrophages filled with haemoglobin. Methyl green-pyronine.  $\times 100$

After 20 days considerable parts of the transplants had been absorbed. Their whole area was so densely interwoven with capillaries that frequently nothing but the vascular network was observable. There were many macrophages, filled with haemoglobin, also many plasmocytes. No intact hepatic structure was visible.

(iv) *Transplantation of regenerative liver to the anterior chamber of rats with impaired liver*

The general picture ten and twenty days after transplantation was as under (ii).

### Discussion

According to the findings, the process of regeneration seems to be favourable for the survival of transplants. Whether it was in the transplanted tissue or in the liver of the recipient animal that regeneration was under process, transplantation was more successful than in cases where intact tissue was transferred into intact hosts. There were, nevertheless, some differences between the particular forms of transplantation which may throw light upon certain biological laws.

The attempt to transplant normal hepatic tissue into rats with an intact liver was a failure. The highly differentiated liver tissue usually does not survive explantation on heterologous medium [2], although immunological

factors do not harm it in tissue cultures. The possibility of immune reactions is commonly regarded as limited in case of implantation into the eye-chamber [9, 10], although, according to both the literature [6] and our own experience, injury to the eye opens the path to immune reactions, a fact confirmed by the appearance of plasmocytes. Destruction was complete in our experiments, but this could not be ascribed to immunological factors alone, considering the different fate of normal tissue transplanted into animals with intact and animals with a damaged liver. Not only did the transplant in hosts with impaired liver survive the first ten days, but it was alive even on the 20th day and seemed, moreover, to have developed in the meantime. It is evident that, while the immunological factors were identical in both cases, humoral-regenerative factors produced in the latter case an effect promoting the vitality of the implanted tissue.

The primary role of regenerative factors was further illustrated by the fact that although transplants obtained from donors with an injured liver (*i. e.* regenerative tissues) were on the 10th day still viable and presented a picture similar to that described in the foregoing, they showed marked signs of degeneration and began to be absorbed by the 20th postoperative day. This observation proves that regenerating hepatic tissue, if transplanted, has a decidedly higher vitality than unimpaired tissue but needs a permanent stimulus for its survival, a stimulus ensured by the regenerative liver of the host or rather those active substances which are released by it.

Our experimental results seem to support our theory of the three phases. The fact that, aided by the regenerative factor, the transplant survived the immunologically critical 8th day and that the vascularized tissue was still alive on the 20th day, bears out the significance of the second phase. It should be borne in mind that both regenerative and intact hepatic tissue provoke an immune reaction. The fact that more decomposed tissue induce more intensive immune reactions does not diminish the significance of the phase of assimilation and adaptation, since the very process of decomposition is due to disturbed assimilation. It is thus obvious that the humoral and, to a slighter extent, the local actors of regeneration promoted the success of transplantation by enhancing the vitality of the implanted tissue and possibly also by promoting its differentiation.

The identical behaviour of homologous and heterologous transplants shows that if a highly differentiated tissue like the liver of adult mammals is transplanted, the success or failure of the operation depends much more on the degree of differentiation, *i. e.* the vitality of the transferred tissue, than the racial difference between donor and host [4, 5].

### Summary

Homologous and heterologous normal and regenerative hepatic tissue has been transplanted into rats with intact and rats with damaged liver. Survival of the transplants was promoted by a regeneration of the recipient's liver and also but less, by that of the donors. The cause of the phenomenon and its connection with the three-phase theory have been discussed.

### REFERENCES

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### ТРАНСПЛАНТАЦИЯ ПЕЧЕНОЧНОЙ ТКАНИ В УСЛОВИЯХ РЕГЕНЕРАЦИИ

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На крысах с непораженной и с пораженной печенью проводились гомо- и гетеротрансплантации ткани здоровой печени крыс или же печени в стадии регенерации, причем было установлено, что регенерация печени реципиента выражено, а регенерация печени донора в меньшей степени способствует приживанию трансплантатов. Обсуждаются причины этого явления, или же его связь с трехфазовой теорией.

### ÜBER DIE TRANSPLANTATION VON LEBERGEWEBE IM REGENERATIONSSTADIUM

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An Ratten mit intakter und geschädigter Leber wurden Homo- bzw. Heterotransplantationen von normaler Rattenleber bzw. von regenerierender Rattenleber durchgeführt. Es wurde festgestellt, daß die Regeneration der Leber des Rezipienten in stärkerem, die Regeneration der Leber des Donors hingegen in geringerem Maße das Überleben der Transplantate fördert. Die Ursachen dieser Beobachtung, bzw. ihr Zusammenhang mit der Dreiphasen-Theorie werden diskutiert.

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