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LIVER HETEROTRANSPLANTATION EXPERIMENTS. TEST OF THE THREE-PHASE THEORY ON DIFFERENT TISSUES OF THE SAME ORGAN

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Earlier experiments led us to the conclusion that there exist three phases in the life of heterotransplants [1]. The first is the phase of local nutritional disturbances, and these alone suffice to destroy many kinds of tissue. The second phase is that of assimilation and adaptation which heterotransplanted tissues cannot survive unless they are able to utilize foreign proteins. In the third phase, the transplant comes to be exposed to the immune-biological reaction of the recipient organism. The second phase appears to determine the destiny of heterotransplants both in a positive and a negative sense : tissues capable of surviving this phase are also able to resist the immune reaction occurring in the third phase ; however, the major part of tissues is unable to utilize foreign proteins so that the immune substances produced during the third phase usually attack cells that are dead already. In the course of the experiments which led to these conclusions splenic and hepatic tissue, taken from phylogenetically different animals, had been transplanted [2, 4, 5], and the conclusions were a result of a comparison between the outcome of these experiments and our observations regarding the behaviour and nutritional conditions of phylogenetically and ontogenetically different tissues in various kinds of tissue cultures [3]. Yet, we possess but scanty data regarding the question as to how far the said three-phase theory remains valid if applied to tissues which, although forming parts of one and the same organ, are nevertheless different in respect of both vitality and the degree of their ontogenetic development. To find an answer to this question was the object of the present series of experiments.

Methods

After injuring the liver of adult guinea pigs with scissors, the liver of 17-day old rat embryos was transferred to it. The implanted pieces of hepatic tissue had a size of 2 mm^3. The wound of the recipient liver was tamponed with cotton saturated with Thrombofort (Richter). The abdominal wall of the host was closed under sterile conditions. The heterotransplants and the surrounding host tissues were removed — likewise with all precautions of sterility — 2, 4, 24 hours, 2 and 8 days after the operation. The transplant was sharply distinguishable from its environment on account of its extremely pale colour as early as 2 hours after the operation. Separating the transplant and the surrounding host tissues in the

excised pieces, we placed both of them in tissue cultures. We used partly Maximow's hanging-drop and partly roller-tube cultures ; the nutrient medium was fowl plasma clotted with chick-embryo extract. The washing fluid was a 3:1:6 mixture of horse serum, chick-embryo extract and Tyrode solution.

The length of cultivation, and also that of observation, was 8 days, i. e. 192 hours. Only epithelial proliferation was accepted as growth in the cultures.

The second series of investigations differed from the first only in that the donors were not embryonic but adult rats.

The tissue transferred to the cultures was always taken from the central zone of the transplants, while the explanted substances of the environment were obtained from the hepatic portion of the host adjacent to the margin of the explant.

A total of 50 animals was used as tests and 1200 tissue cultures were studied in the course of the experiments.

Experimental and discussion

Transplantation of embryonic liver

The results of the experiments are collected in Table 1 and illustrated in Fig. 1.

Table 1

Percentage of cultures displaying growth of explanted embryonic liver

Age of explants	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h
Explanted 2 hours after transplantation	Transplant	—	16	16	16	16	16	19
Explanted 2 hours after transplantation	Surrounding tissues of g. pig liver	—	—	—	—	—	—	—
Explanted 4 hours after transplantation	Transplant	—	16	16	16	16	16	19
Explanted 4 hours after transplantation	Surrounding tissues of g. pig liver	—	—	—	—	—	—	—
Explanted 24 hours after transplantation	Transplant	6	6	6	45	50	50	50
Explanted 24 hours after transplantation	Surrounding tissues of g. pig liver	—	12	12	75	75	75	75
Explanted 48 hours after transplantation	Transplant	—	—	—	53	61	61	61
Explanted 48 hours after transplantation	Surrounding tissues of g. pig liver	—	12.5	12.5	71	100	100	100
Explanted 8 days	Transplant	—	—	32	32	32	32	32
Explanted 8 days	Surrounding tissues of g. pig liver	—	18	67	67	67	67	72

If we explant embryonic liver, fibroblastic proliferation can be observed after 24 hours ; after another 24 hours epithelial growth sets in which assumes considerable dimensions 3 days after explantation. The picture is — as can be seen from our experiments — entirely different if the embryonic liver is transferred to the same organ of a heterologous animal : no, or only very feeble, growth takes place during the first two or four hours, and the histological picture of the material fixed at this time reveals destroyed hepatic

parenchyma cells at every point ; stained with methyl green pyronine, it is only the epithelium of the small bile ducts which shows pyroninophilia. What do these phenomena signify? That, in the first phase of heterotransplantation, the different tissues of the transplanted organ-fragment respond to the trauma

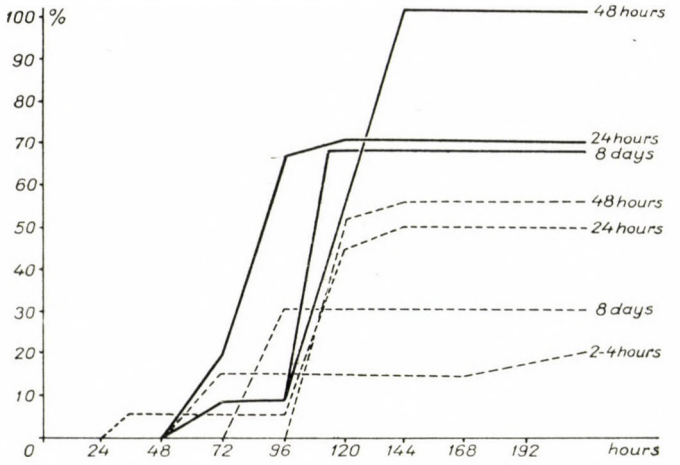


Fig. 1. Explantation of embryonic liver transplants to tissue cultures. Solid lines signify growth of the surrounding guinea-pig liver, broken lines that of the transplants

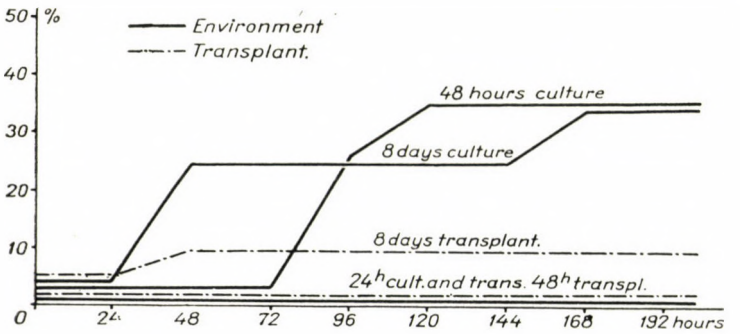


Fig. 2. Explantation of adult liver transplants to tissue cultures. Solid lines signify growth of the surrounding guinea-pig liver, broken lines that of the transplants

differently. It would be erroneous to suppose that the embryonic liver parenchyma, known to thrive vigourously on heterologous media in tissue cultures, had degenerated in our experiment because of having failed to assimilate alien proteins. If for no other reason, such assumption would be erroneous on account of the fact alone that every cell carries a certain amount of residual energy which is in any case sufficient to prevent damage attributable to lack of nutrition during the first 2 or 4 hours following transplan-

tation. That nutritional factors had nothing to do with the observed destruction of the cells in borne out by the fact that — when fragments of liver, corresponding in size to the implanted ones, were placed in Tyrode solution and put in a 37° C thermostat for 2 and 4 hours — the explanted pieces continued to proliferate at a rate which was hardly inferior to that of the controls (Fig. 3).

The conclusion which, therefore, presents itself is that, while the blood and bile escaping at the traumatization of the liver of the recipient guinea

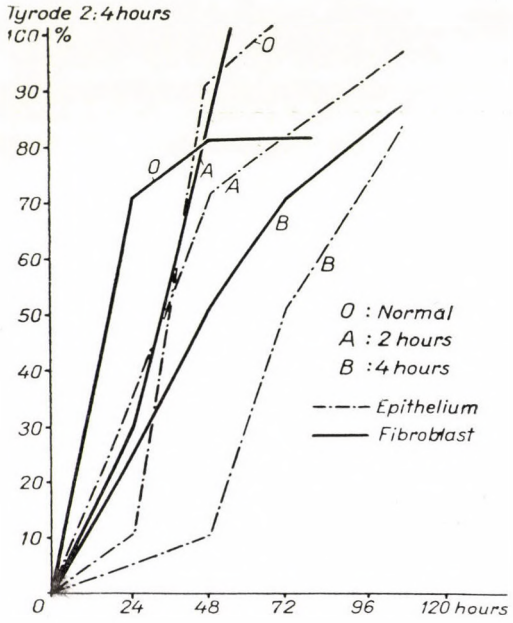
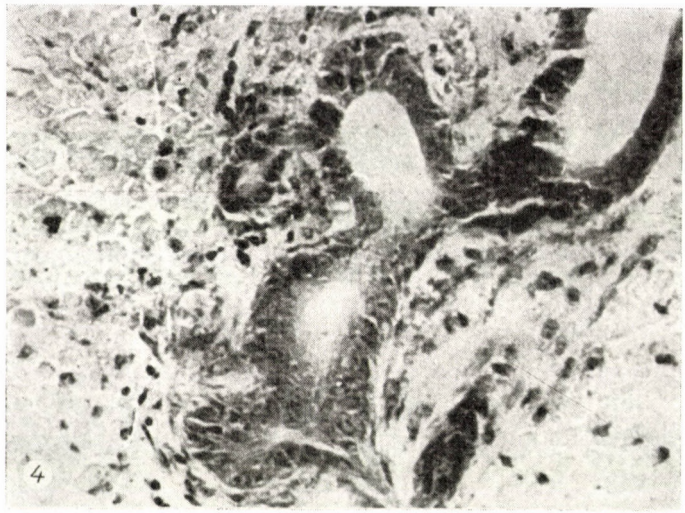


Fig. 3. Explantation of embryonic livers kept in Tyrode solution

pig did serious damage to the hepatic parenchyma, the small bile ducts displayed more resistance and managed to survive. Having thus passed unscathed the first phase that had proved fatal to the hepatic parenchyma, the bile ducts came into the second phase after the lapse of 24 to 48 hours in which they had to adapt themselves to the heterologous environment and assimilate the foreign proteins [6]. 50 to 55 per cent of our transplants were found to grow in this phase, a dependable sign of their having been able to utilize foreign proteins, a *conditio sine qua non* of their surviving the second phase.

Serial sections of the transplants presented a very interesting picture. While not a single intact parenchyma cell could be detected and none of them betrayed the least trace of pyroninophilia, we found among the entirely degenerated parenchyma cells intensely pyroninophilic and vigourously proliferating small bile ducts (Fig. 4).

Their growth was of a type different from that of normally proliferating embryonic liver parenchyma. The growth of the latter usually sets in over an extended area : the epithelial membrane grows almost over the entire



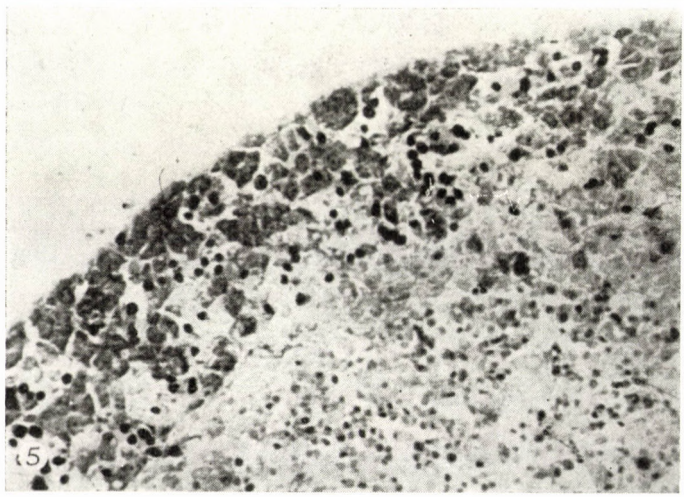


Fig. 4. Histologic picture of explanted embryonic liver transplant. 100× Methyl green pyronine
Fig. 5. Histologic picture of explanted embryonic liver. Control. 100× Methyl green pyronine

surface of the culture, while the histological picture presents necrosis in the central and densely packed parenchyma cells in the marginal zone (Fig. 5).

Against this picture, the growth of the small bile ducts is characterized by the appearance of small epithelial spicules at various points of the culture's

surface : they attain quite a considerable length and spread after 2 to 3 days over the whole surface and the zone of migration. After the lapse of 3 to 4 days it is only by means of serial sectioning of the culture that one can distinguish the epithelial growth of the small bile ducts from that of the hepatic parenchyma.

The fact that the proliferation of the small bile ducts could be observed also in cultures to which the transplants had been transferred 8 days after transplantation seems to prove that, transplanted to a heterologous medium, the epithelial cells of the bile ducts respond differently from those of the hepatic parenchyma. Local traumatization destroys the latter as early as the first phase of transplantation so that it cannot be observed in the subsequent phases. Not so the epithelium of the small bile ducts. Having survived and even started to proliferate during the first phase, it assimilates foreign proteins during the second phase, and retains its vitality even after the immunebiologically critical first week. All this shows that the theory regarding the three phases remains valid also if applied to the different tissues of one and the same organ. The assimilative readiness of the ontogenetically younger tissue helps it to survive the immunebiologically critical phase.

Transplantation of adult liver

The results of these experiments are summarized in Table 2 and illustrated in Fig. 2.

Table 2

Percentage of cultures displaying growth of explanted adult liver

Age of explants	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h
Explanted 24 hours after transplantation	Transplant	—	—	—	—	—	—	—
Explanted 24 hours after transplantation	Surrounding tissues of g. pig liver	—	—	—	—	—	—	—
Explanted 48 hours after transplantation	Transplant	—	—	—	—	—	—	—
Explanted 48 hours after transplantation	Surrounding tissues of g. pig liver	—	—	25	37.5	37.5	37.5	37.5
Explanted 8 days	Transplant	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Explanted 8 days	Surrounding tissues of g. pig liver	25	25	25	25	25	25	37.5

Table 2 and Fig. 2. make it evident that transplanted adult livers behave differently from embryonic ones. While, after 24 and 48 hours, no proliferation can be observed in the transplant itself, the 48-hour old surrounding host tissue is steadily growing. It is therefore obvious that proliferation which,

subsequently, spreads over the zone of transplantation as well, is caused by the small bile ducts spreading from the tissues that surround the zone of transplantation, and it must be assumed that both kinds of tissues of the transplanted adult liver succumb to traumatization and the assimilative-adaptive disturbances.

Summary

Experiments have been made with a view to testing the correctness of the theory of three phases in the life of heterotransplants in its application to various tissues of the same organ. Embryonic and adult rat livers were transplanted to the liver of adult guinea pigs ; after explanting the tissues, the growth of the parenchyma and the small biliary ducts was studied. It was found that — within one and the same organ (liver) — the parenchyma degenerated in the first phase, while the small ducts survived it. The latter continued to live and even proliferate during the second phase also. The experiments allow the conclusion that the three-phase theory holds good even if applied to different tissues of one and the same organ.

REFERENCES

1. Csaba, Gy.: (1956) Three phases in the life of heterotransplants. Acta Biol. Hung. *6*, 361—364. — 2. Csaba, Gy. Iskum, M.: (1958) Heterotransplantation von Milzgewebe nach vorangehender Adaptation in der Gewebekultur. Acta Morph. Hung. *8*, 71—76. — 3. Csaba, Gy., Cs. Hegyi Klára : (1958) Behaviour of phylogenetically and ontogenetically different tissues in tissue cultures explanted on autologous, homologous and heterologous media. Acta Biol. Hung. *8*, 149—164. — 4. Csaba, Gy., Iskum, M.: (1958) Heterotransplantation of spleen taken from phylogenetically different animals. Acta Biol. Hung. *8*, 253—262. — 5. Csaba, Gy.: (1958) Die Rolle der Assimilations-Adaptationsfaktoren in der Entwicklung von Heterotransplantaten. Virchow's Arch. (to be published). — 6. Ultmann, L., Hirschberg, C., Gellhorn, K.: (1953) The effect of nitrogen mustard on the cellular concentrations of nucleic acids in regenerating liver. Cancer Res. *13*.

ОПЫТ ПО ГЕТЕРОТРАНСПЛАНТАЦИИ ПЕЧЕНИ. ИССЛЕДОВАНИЕ ТРЕХФАЗНОЙ ТЕОРИИ В ОТНОШЕНИИ РАЗЛИЧНЫХ ТКАНЕЙ ОДНОГО И ТОГО ЖЕ ОРГАНА

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Авторы исследовали правильность трехфазной теории гетеротрансплантации в отношении различных тканей одного органа. Проводилась трансплантация печени эмбриональных и взрослых крыс в печень взрослых морских свинок; затем ткани выращивались в культурах и исследовалась способность роста паренхимы и желчных путей. Авторам удалось установить, что внутри одного и того же органа (печень) паренхима в первой фазе погибает, в то время как желчные пути дальше существуют. Желчные пути существуют также и во второй фазе и приобретают способность роста. На основе своих исследований авторам удалось доказать, что трехфазная теория имеет действие также в отношении различных тканей одного органа.

HETEROTRANSPLANTATIONSVERSUCHE MIT RATTENLEBERN. UNTERSUCHUNG DER DREIPHASENTHEORIE HINSICHTLICH DER VERSCHIEDENEN GEWEBE DES GLEICHEN ORGANS

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Die Richtigkeit der Dreiphasentheorie der Heterotransplantation hinsichtlich verschiedener Gewebe eines Organs wurde untersucht. Die Leber von embryonalen und erwachsenen Ratten wurde in die Leber von erwachsenen Meerschweinchen transplantiert; hiernach wurden die Gewebe in Kulturen verpflanzt; und die Wachstumsfähigkeit des Parenchyms und der Gallenwege untersucht. Es gelang festzustellen, daß innerhalb des gleichen Organs (Leber) das Parenchym in der ersten Phase zugrunde geht, während die Gallenwege fortbestehen. Die Gallenwege existieren auch noch in der zweiten Phase und erwerben Wachstumsfähigkeit. An Hand des Versuche wird der Nachweis erbracht, daß die Dreiphasentheorie auch in bezug auf die verschiedenen Gewebe eines Organs Gültigkeit hat.

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