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The Cellular Reaction of the Kidney After Different Physical Injuries*

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Summary. Autoradiographic investigations on kidney cells were performed after focal cryolesions (-180 to -196°C) and focal heat application (740°C). Cells were studied 12 h to 30 days after the lesions had been produced. In the damaged granulation tissue the percentage of radioactively labelled fibroblasts as well as the percentage distribution of leucocytes, monocytes, macrophages, lymphocytes, fibroblasts and fibrocytes and the mean cell concentration were determined.

There were no significant differences in the leucocytic and monocytic cell reactions after the two types of physical injuries. However, the percentage of fibroblasts, fibrocytes and macrophages was higher and the percentage of lymphocytes lower after cryonecrosis when compared to heat application. The cell concentration increased during the last 2 weeks of the experimental time after a thermolesion. The labelling index of the wound fibroblasts was significantly higher after the 10th day after thermonecrosis than after in situ freezing.

The increased cellular activity 2 to 4 weeks after heat coagulation of the kidney was probably induced by the delayed resorption of the carbonised necrotic tissue. The reduced phagocytic activity of macrophages might have depended on alteration and modification of molecular cell structures which were different after heat application or freezing.

The different lymphocytic reaction seems to be the consequence of different immune responses of the lymphatic system. It is suggested that focal thermolesions may have a stimulatory effect on the cell-mediated immune response and that focal cryonecrosis may induce an increase in humoral immune response.

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Key words: Heat coagulation, in situ freezing, Tissue repair, Kidney.

INTRODUCTION

Morphological studies of wound healing in the kidney may be made after a variety of different injuries-mechanical, thermal and cryolesions.

Extensive studies of the morphological changes after needle biopsies of the kidney are available (5, 6, 7, 8).

The dynamics of wound healing in the kidney after focal heat or cryolesions have also been investigated using ^{3}H-thymidine autoradiography (12, 13). Comparing the changes after cryo- and thermo-lesions it was found that wound healing, and especially the resorption of thermonecrotic material, was delayed (4, 13). As a possible cause of the delayed wound healing after focal heat application a more severe tissue destruction has to be considered. However, it is also possible that after these physical injuries cellular activities with different resorptive capacities develop. In order to answer these questions, comparative cell-analytical investigations were performed on the granulation tissue developing after focal heat and cryolesions on the kidney of rats.

MATERIAL AND METHODS

After laparotomy the left kidney of 60 previously untreated Wistar rats (average wt 180 g) was locally frozen with a cryoprobe (H 29 Grünenthal-Frigitronics; Cryocauter 190) with a diameter of 6.5 mm (temperature of the cryoprobe -180 to 196°C; freezing time 30 s. [1]

[1] Parts of the cryoexperiments were performed together with H. Breining and S. Lymberopoulus (2, 3)

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A further 40 rats (average wt 189 g) received focal heat coagulation of the left kidney (740°C/ 4 s). The corresponding tissue area also measured 6.5 mm in diameter.

The postoperative survival time varied from 12 h to 30 days. One hour before sacrifice each animal received an intraperitoneal injection of ^{3}H-thymidine (2.5-3.0 µCi/1 g body weight, specific activity 20.0 Ci/mmol, NEN Chemicals, Boston, Massachussetts, USA). After sacrifice the damaged kidneys were removed, fixed in formol and embedded in paraplast. Stripping film autoradiograms (AR 10, Kodak) were made in the usual manner. Exposure time was 21 days. The histological slides were stained with haematoxylin eosin, PAS; and after Giemsa and van Gieson. Using a graticule inserted ocular (Leitz) the various percentages of polymorphonuclear granulocytes, monocytes, macrophages, lymphocytes, fibroblasts and fibrocytes as well as multinuclear giant cells of the foreign body type were determined in the growing granulation tissue following the thermo- or cryo-necrosis. Furthermore, the cellular concentration for each of the corresponding cell populations and the mean cell number per unit tissue area (0.0083 mm^2) were evaluated. At least 3,000 cells per slide were analysed. From the autoradiographs the percentages of the radioactively labelled fibroblasts in the granulation tissue were determined. The standard deviations of the depicted means correspond to 2 s.

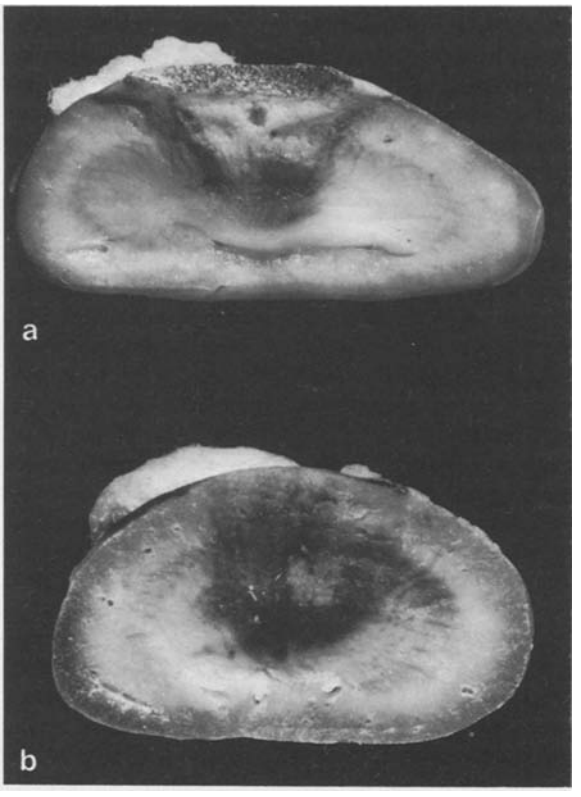

Fig. 1a, b. a Tissue lesion 24 h after thermocoagulation of the kidney. b Tissue lesion 24 h after in situ freezing

RESULTS

Macroscopic Findings

After the removal of the cryoprobe and cessation of the heat coagulation, wedge-like tissue necrosis developed within the first 12 to 24 h, surrounded by a more or less distinct haemorrhagic margin (Fig. 1a, b). Ten to 14 days after heat or cold injuries only a small zone of granulation tissue was seen on the surface of the kidney after in situ freezing, whereas necrotic material with resorptive inflammation was observed for 4-6 weeks after heat coagulation (Fig. 2a). Four weeks after freezing, however, a fibrous scar indicated the end of the wound healing (Fig. 2b). A corresponding picture was found 8 weeks after heat coagulation.

Histologically, 12 h after the freezing or heat coagulation only shadow-like glomerular and tubular structures were recognisable (Fig. 3a, b). Around the organ capsule and in the marginal zones between the vital and damaged tissue a sparse granulation tissue developed. In the central parts of the cryonecrosis a strange tissue hyalinisation was visible.

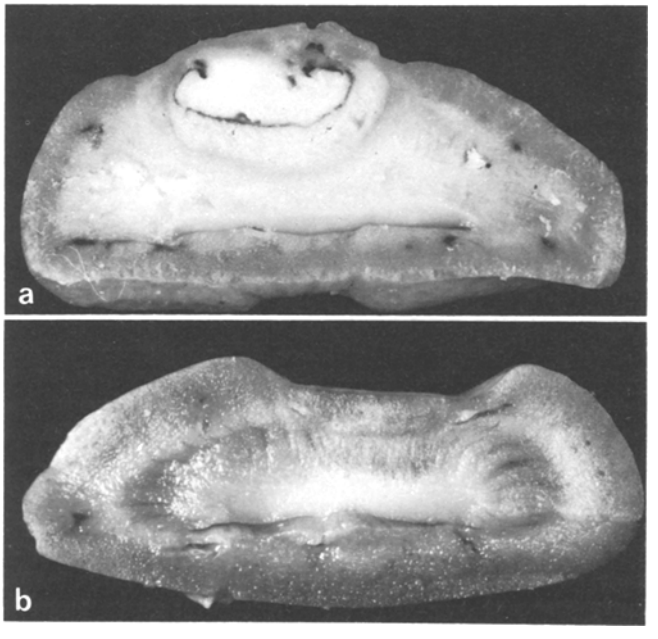

Fig. 2a, b. a Thermonecrosis 4 weeks after heat application with inflammation. b Fibrous scar 4 weeks after cryonecrosis of the kidney

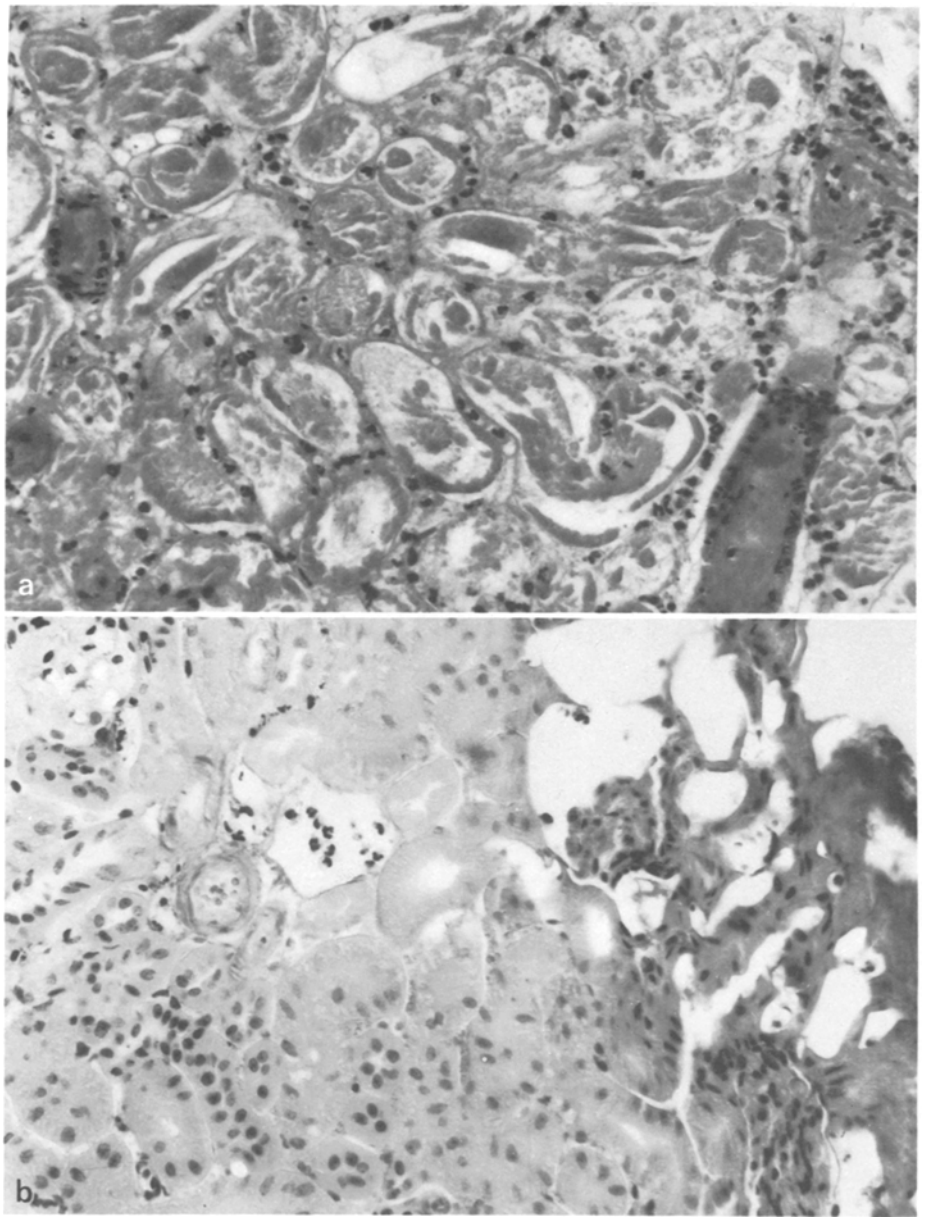

Fig. 3a, b. a Cryonecrosis of the kidney with leucocytes 12 h after freezing. b Necrotic tissue of the kidney with carbonized material on the surface 12 h after heat coagulation (HE, 310 x)

After day 10 of freezing, distinct microcalcifications were seen in the necrotic tubulus area. These calcareous deposits were later surrounded by round cell infiltrates and multinuclear giant cells of the foreign body type (Fig. 4a). In the thermocoagulated area of the kidney multinuclear giant cells were observed at the same time which had built up around carbonised material (Fig. 4b). After both lesions a distinct retraction of the necrosis had taken place after 10 to 20 days to about 2/3 of the original volumes.

However, 4 weeks after in situ freezing only a calcified, relatively acellular fibrous scar was observed and larger amounts of carbonised necrotic tissue were still found after focal heat coagulation which had not been completely resorbed. At the transition to the preserved kidney tissue a distinct resorptive inflammatory reaction prevailed (Fig. 5).

Cell Analysis

In Fig. 6a, b the percentage distribution of the different cells in the granulation tissue at different intervals after both types of physical damage are shown.

On days 1-3 after both cryo- and thermolesions polymorphonuclear leucocytes prevailed. After day 10 these cells did not play an important part after either type of injury.

The activity of fibroblasts was more prominent after thermonecrosis during the first 3 to 4 days than after a cryolesion. However, after day 5

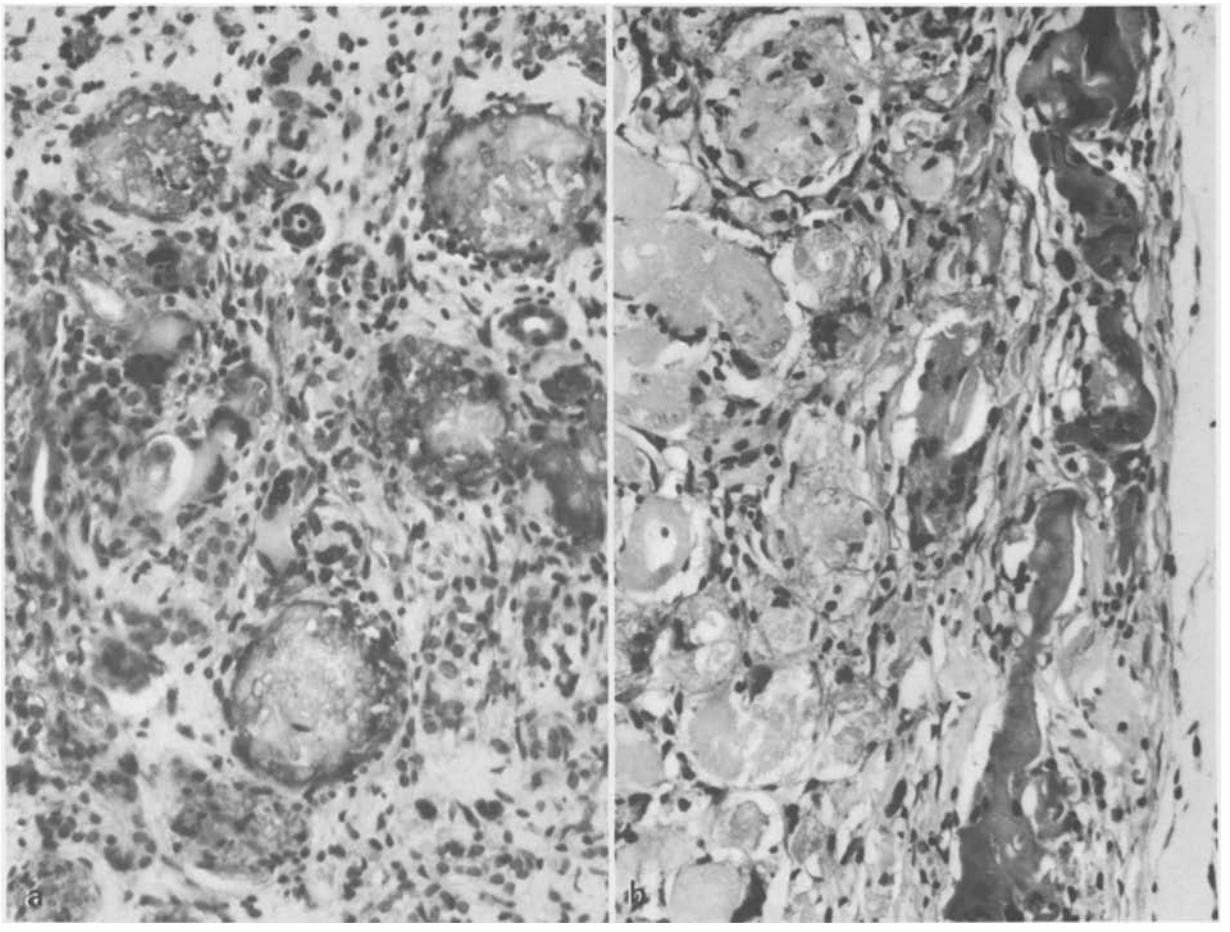

Fig. 4a, b. a Microcalcifications in the kidney parenchyma 3 weeks after in situ freezing. b Multinuclear giant cells around carbonised tissue of the kidney 3 weeks after heat application

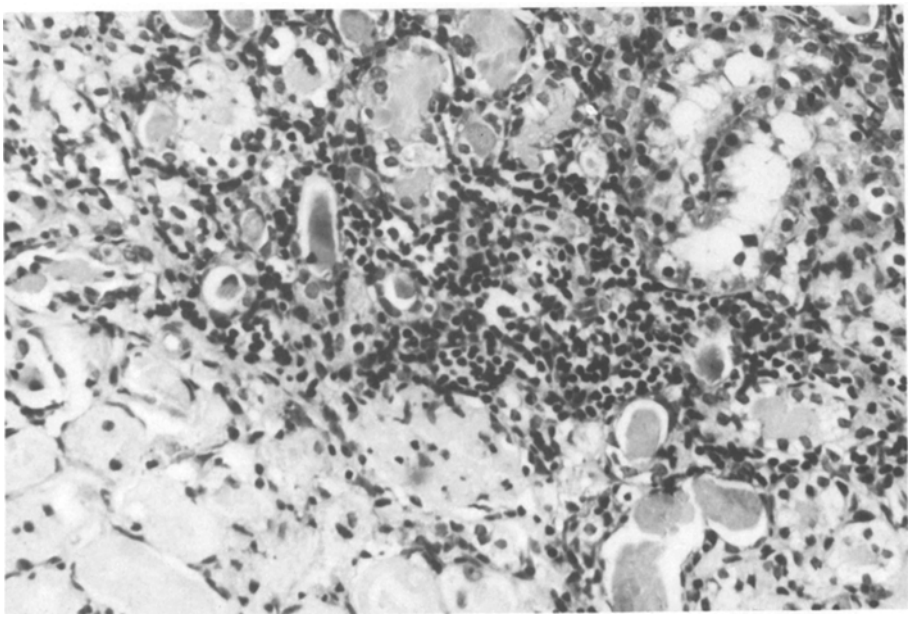

Fig. 5. Resorptive inflammation in vicinity of heat damaged tissue of the kidney 4 weeks after thermonecrosis

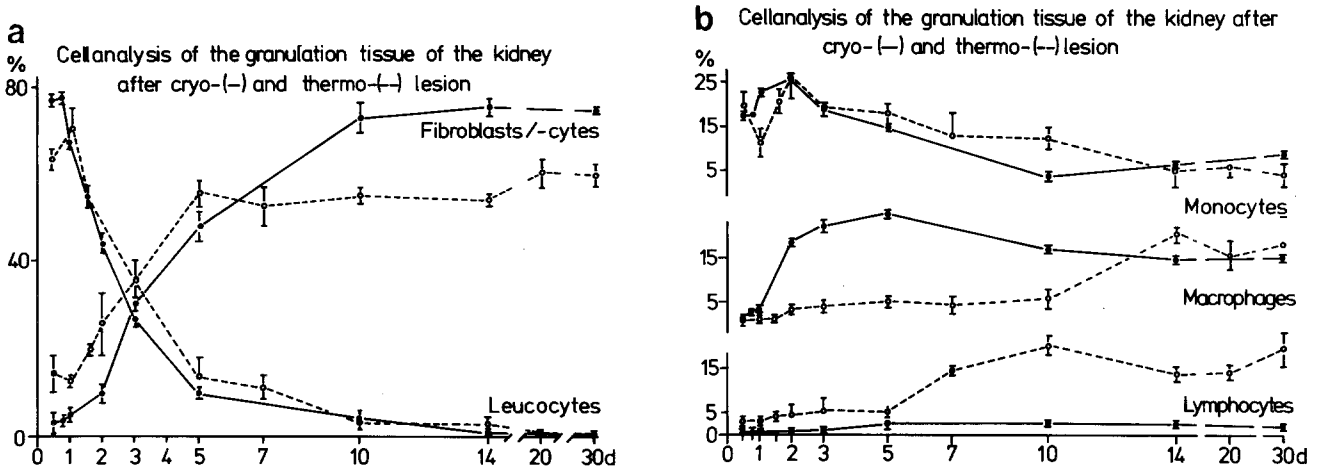

Fig. 6. Cellular analysis of the granulation tissue after focal thermonecrosis (– – – –) and cryonecrosis (————) of the kidney. a Percentages of fibroblasts/fibrocytes and leucocytes. b Percentages of monocytes, macrophages and lymphocytes

the percentage of fibroblasts was still distinctly lower than after a single cryonecrosis.

The percentage of monocytes was not significantly different after the 2 kinds of injury. Macrophage numbers were significantly higher up to day 10 following focal cold application.

The lymphocytes showed a reversed percentage distribution with significantly higher percentages after day 5 following the thermonecrosis. The percentages of multinucleated giant cells found were not significantly different.

Cell Concentration

The determination of the number of different cell types per 0.0083 mm^2 tissue area confirmed the results of the percentage distributions during the exudative and initial resorptive phases of wound healing after both types of tissue injury. However, after day 10 a gradual decrease in the cell concentration took place after a single cryonecrosis, whereas after a single thermonecrosis the cell concentration in the granulation tissue continually increased, mainly because of an increase of fibroblasts and fibrocytes (Fig. 7).

Cell Kinetic

The cell kinetic investigation showed that the highest proliferative activity of fibroblasts in the granulation tissue of the kidney after both lesions lay between the 2nd and 3rd post-operative day. Between the 2nd and 4th week the percentages of radioactively labelled fibroblasts were significantly higher after thermonecrosis than after cryolesions (Fig. 8).

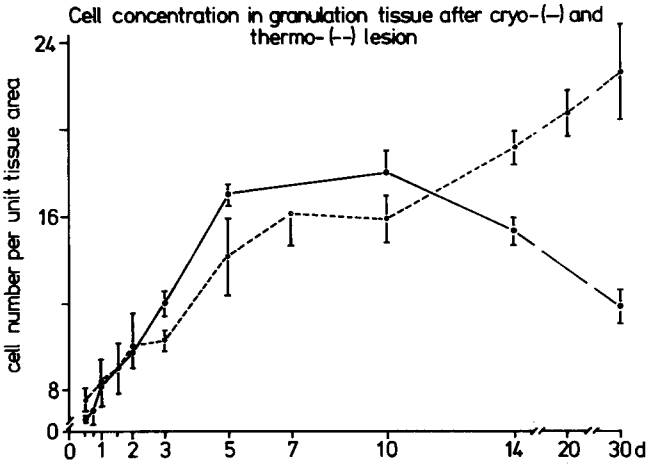

Fig. 7. Mean cell concentration in the granulation tissue (cell number per unit tissue area of 0.0083 mm^2) after cryo (————) and thermo (– – –) lesion

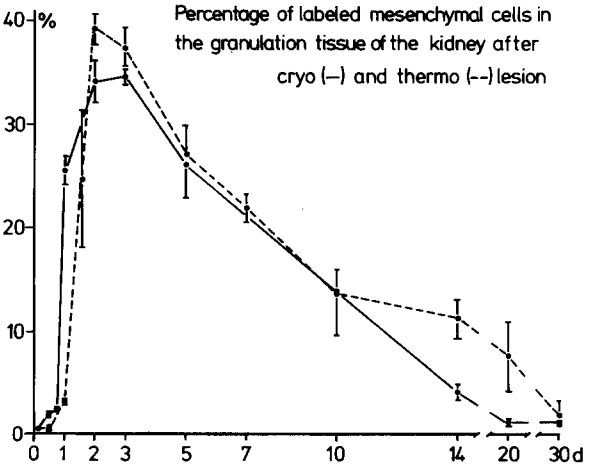

Fig. 8. Percentages of radioactively labelled fibroblasts (labelling index) in the granulation tissue of the kidney after cryo (————) and thermo (– – –) lesion

DISCUSSION

The physico-chemical basis of heat and cold damage to cells, tissues and organs has been described (14, 1, 15). The reparative and regenerative processes have also been investigated by cell kinetics using ^{3}H-thymidine autoradiography. Differences in the time of the maximal proliferative activity of both mesenchymal and epithelial cells 2 and 3 days after in situ freezing or heat coagulation have not been observed before. However, we have found that proliferative activity of epithelial cells after cryonecrosis is higher than after thermonecrosis. We also found that after thermonecrosis tissue bridges or vascular septi are not present to the same degree as after cryolesions; evidence of healing was less apparent after a single thermonecrosis. However, the most significant result of this investigation is the observation that 2 to 4 weeks after the application of heat more distinct proliferative activity of fibroblasts, with increasing cell concentration in the granulation tissue, is visible. This contrasts with a single in situ freezing after which the cell concentration decreases and the labelling index of the wound fibroblasts shows lower values. This late cellular activity after thermocoagulation may be induced by a delayed resorption of the carbonised necrotic material in the kidney. The cellular analysis of the granulation tissue underlines this observation. The phagocytic activity of macrophages after in situ freezing is significantly increased. From the 10th post-operative day fibrocytes dominate the morphological picture of the resorptive granulation tissue and the scar. After thermonecrosis, however, the macrophage activity seems to be reduced during the first 10 days. Around the carbonised material multinuclear giant cells are found as evidence of an obviously difficult resorption of thermonecrotic tissue. The reduced phagocytic activity of macrophages depends possibly on alterations and modifications of molecular cell structure which may be different after heat application from that after freezing and therefore the thermodamaged tissue areas may only be resorbed with difficulty by macrophages. The inability of the macrophages to prepare the carbonised material for optimal resorption may play an important part (17).

The relatively high percentages of lymphocytes in the granulation tissue after thermonecrosis may be explained by the observation that only small burns, as was the case in our experiments, exert a stimulatory effect on the cell mediated immune response (16). Accordingly, an increase in the number of DNA-synthesising lymphocytes in the thymus was observed after focal thermolesions on parenchymal organs (9). It may be possible that an increased and longer-lasting migration of T lymphocytes to the site of the thermonecrosis takes place as the equivalent of a cell-mediated immune defence reaction against altered carbonised tissue components. On the other hand morphological changes suggesting an increased humoral immune response can be induced in the spleen after focal freezing of parenchymal tissues (10).

As in other organs, tissue reparation after focal cryonecrosis in the kidney is shorter and less complicated than after thermocoagulation. But on the whole, the differences in the dynamics of wound healing after cryo- and thermolesions are not as distinct as in the liver (11).

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