From:
The Department of Anatomy,
Dental Faculty,
University of Oslo, Norway

# THE FATE OF RESORBABLE HEMOSTATIC IMPLANTS IN RATS

*by*
OLAV HJORTDAL

## INTRODUCTION

The introduction of a gauze-like material with hemostatic properties which could be left *in situ* for the control of venous and capillary oozing has since long been desirable in all fields of surgery. In the field of oral surgery such a preparation would be useful especially for the control of hemorrhage associated with tooth extractions and cyst operations.

Several resorbable hemostatic agents have been proposed: Fragments of raw muscle and fascia as well as organizing blood clots (*Cushing*, 1911), fibrin preparations from animal blood (*Grey*, 1915; *Harvey*, 1918), fibrin foam preparations made from human plasma (*Ferrey & Morrison*, 1944; *Berring*, 1944) oxidized cellulose (*Yackel & Kenyon*, 1942; *Frantz*, 1943), preparations made from gelatin (*Correl & Wise*, 1945), and preparations made from collagen (*Peacock et al.*, 1965).

Preparations made from gelatin and oxidized cellulose seem to be most commonly used to-day.

Preparations made from gelatin are available as a sponge-like material under trade names as Spongostan*) and Gelfoam**). The hemostatic property of the material is supposed to be due to increased liberation of thromboplastin

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on contact with blood because of disruption of platelets in the myriads of pores of the sponge (*Jenkins et al.*, 1946). Gelatin preparations have been used for epistaxis and in the different fields of surgery: Neurosurgery (*Meirowsky*, 1953, 1954; *Heppner & Diemath*, 1962), thoracic surgery (*Bing et al.*, 1951), liver surgery (*Mygind*, 1948), oral surgery (*Thoma*, 1952), and for treatment of carotid-cavernous fistula by embolization (*Ishimori et al.*, 1967). Gelatin sponge has also been used as vehicle for topical application of antibiotics (*Schulte*, 1964) and cytostatics (*Heppner et al.*, 1961).

Preparations made from oxidized cellulose are available as cotton and gauze under names as Oxycel,*) Novocell**) and Surgicel***). On contact with blood, oxidized cellulose is transformed to a dark reddish-brown, tenacious mass which sticks readily to the the wound. The hemostatic property of the material is supposed to be due to its adhesion to the bleeding surface, and because the swelling of the agent probably causes some pressure on the surrounding tissue. Preparations made from oxidized cellulose have been applied in similar cases as preparations made from gelatin: Neurosurgery (*Scarff et al.*, 1949), thoracic surgery (*Cory*, 1946), liver surgery (*Ficarra*, 1950), anorectal surgery (*Anderson*, 1948), oral surgery (*Gwinn et al.*, 1948; *Reid et al.*, 1964).

*Histological investigations*

Compared with the large number of clinical reports on the use of gelatin sponge and oxidized cellulose, histological investigations are relatively few. *Correl* and *Wise* (1945) implanted gelatin sponge into the hind leg muscle of rats, and found by visual and histological examination that the material had disappeared 30 days after implantation. The same findings were made by *Bing* (1947) after subcutaneous and intraperitoneal implantation of Spongostan in rats. *Blomqvist et al.*, (1962) implanted Gelfoam in tooth sockets after extractions in humans, and found by histological investigation of biopsies which were taken out at varying intervals that the material had completely disappeared from the sockets 18 days after implantation.

There are also some reports on histological investigations after implantation of oxidized cellulose. *Frantz* (1943) implanted oxidized cellulose in brain, knee-joint, and peritoneal cavity of dogs and cats. He found the materaial »relatively non-irritating» to the tissues, and it was completely resorbed after 3 weeks. Similar findings were made by *Burns* (1946) after implantation of oxidized cellulose into the peritoneal cavity of dogs and cats. *Frantz* and *Lattes*

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(1945) suggested that oxidized cellulose, because of its acidity, may delay the formation of callus when implanted into clean bone cavities.

At the Department of Oral Surgery and Oral Medicine, Dental Faculty, University of Oslo, the preparations Spongostan and Oxycel were used for some time as inlays in bone cavities after surgical removal of cysts of the jaws. The preparations were treated aseptically and inserted according to prescriptions from the producers, and the wounds were closed by primary sutures. In several cases infection occurred, the cyst cavities had to be reopened, and necrotic remnants of the implanted materials were removed. Because of these unfavourable results the method had to be abandoned, and it was decided to carry out follow-up studies after implantation of the preparations into animals.

## MATERIAL AND METHODS

In order to standardize the experimental conditions, and facilitate the localization of the implanted material on the subsequent histological investigation, the preparations were implanted in cell-permeable millipore chambers. The chambers were modified diffusion chambers which are used for *in vivo* cultivation of cells and tissues (*Algire et al.*, 1954) (Fig. 1). These chambers are well, tolerated by the host animal, and cause no foreign-body reactions (*Algire et al.* 1954). In previous works (*Hjortdal & Rasmussen*, 1969; *Rasmussen & Hjortdal*, 1969) similar chambers, with pore size 0.45 micron, were used for cultiva-

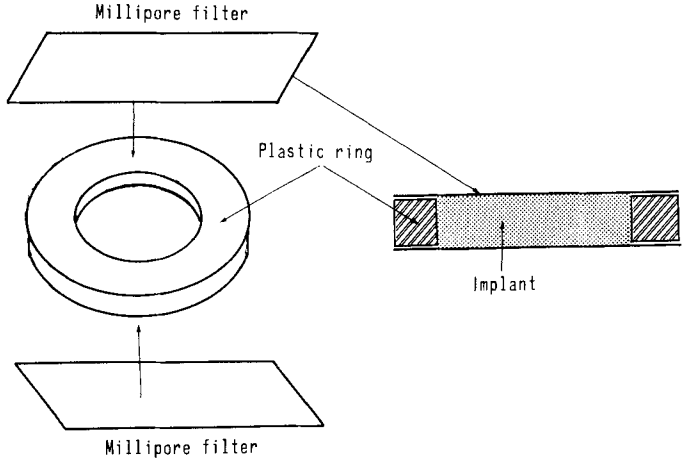



Fig. 1. Diagram illustrating the construction of the millipore chamber.

tion of blood cells. In only 2 out of 112 cases inflammatory tissue reactions adjacent to the chambers were noticed.

The millipore chambers used in the present investigation were constructed from plastic rings with an outer diameter of 13 mm, an inner diameter of 9 mm and thickness 4 mm. The millipore filters were of type NC* with pore size 14 microns $\pm$ 3 microns, and thickness 150 microns. In pilot studies it was found that pores of this size permitted ingrowth of cells as well as capillaries. The chambers were sterilized by dry heat at 75 °C for 24 hours before use. The agents to be investigated, Spongostan and Oxycel, were placed in the chambers together with autogenous blood from the animal's tail. In some cases the agents were moistened with 0.9% aqueous NaCl-solution instead of blood. The chambers were implanted either subcutaneously or intraperitoneally. By subcutaneous implantation Oxycel and Spongostan were placed in the same animal, respectively on the right and left side of the animal's back. Chambers containing only autogenous blood were implanted as controls.

The experimental animals employed were chosen from a stock of hooded rats kept as a closed population in our laboratory. At implantation the mean age of the animals was $2^1/_2$ months, and the mean weight approximately 180 g. The animals were kept in metal mesh cages and were fed a standard diet *ad libitum*. A total of 52 chambers were implanted in 26 animals, 2 chambers in each animal. The surgical intervention was done under Nembutal-Na anesthesia, given intraperitoneally, 0.05 ml/100 g. body weight. The chambers were placed intraperitoneally through an incision in the abdominal wall which subsequently was closed in layers by supramid sutures. Subcutaneous implantation was achieved through an incision on the animal's back, and by dissecting a pouch under the skin on each side of the incision. The operation field was shaved before the incision, and the surgical procedure was done under as strict aseptic conditions as possible.

The animals were killed and the chambers removed for histological investigation at periods: 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 2 months.

*Histological preparation*

The chambers with contents and surrounding connective tissue capsules were fixed for minimum 20 hours in a buffered, neutral, 4% formaldehyde solution. After fixation the implants together with the millipore filters were dissected free from the acrylic rings, embedded in paraffin, and cut at 6 microns. The sections were stained with hematoxylin and eosin, and with Goldner's modification of Masson's trichrome stain.

*) Millipore Filter Corporation, Bedford, Massachusetts.

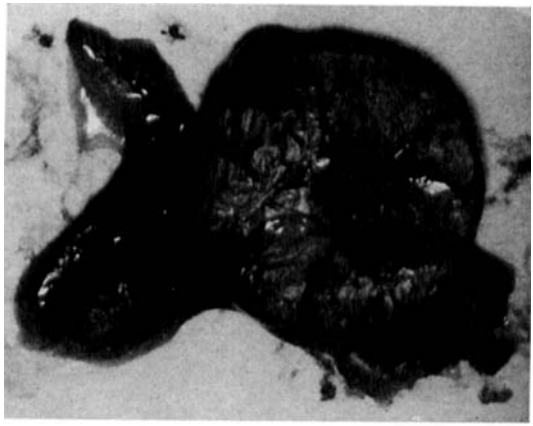


Fig. 2. Millipore-chamber 2 weeks after implantation in the peritoneal cavity. The chamber is enveloped by well vascularized connective tissue.

## RESULTS

At removal the chambers were surrounded by well vascularized connective tissue, which strongly adhered to the filter membrans (Fig. 2). The formation of pus around the chambers was never observed. In 3 cases some serous exudate was found adjacant to the chambers.

### *Microscopic observations*

*Chambers filled with Spongostan and blood.* The contents of the 1-week-old chambers consisted of meshes and strands of the gelatin sponge, embedded in coagulated blood (Fig. 3). Collections of leukocytes were seen surrounding the implanted material. Adjacent to the filter side there was a border of fibroblasts and collagen fibres (Fig. 4). Two weeks after implantation heavy accumulations of mononuclear cells were seen in the pores of the implanted material. Three weeks after implantation a great proportion of the implanted material had disappeared from the chambers (Fig. 5). The leukocyte reaction had subsided; around remnants of unreasorbed gelatin, lymphocytes and macrophages as well as many foreign body giant cells were seen. In the 4 and 5-week-old chambers there was a gradual diminishing of the implanted sponge. However, in some of the chambers considerable amounts were still left. Unresorbed rests were surrounded by cell-rich granulation tissue. Two months after implantation the chambers were for the greater part filled with connective tissue in which several capillaries were seen. There were still remnants of unresorbed sponge which were surrounded by macrophages and mononuclear cells (Fig. 6).

*Chambers filled with Oxycel and blood.* One week after implantation there was a very strong cellular reaction to the implanted material, with heavy accumulations of leukocytes and plasma cells (Fig. 7). After 2 weeks, ingrowth of connective tissue with many capillaries was seen adjacent to the filter side. In the central part of the chambers there were abundant inflammatory cells with degenerative changes. In several areas the cellular picture had the morphological characteristics of pus.

Three weeks after implantation there was still a strong cellular reaction and accumulations of disintegrated cells adjacent to the implanted material, which, in some instances, seemed to have condensed into solid masses. In the 4 and 5-week-old chambers the ingrowth of connective tissue was progressing, but

Fig. 3. Section of chamber containing Spongostan + blood placed intraperitoneally for 1 week. The implanted gelatin sponge (s) is seen between the millipore filters (mf) having the appearance of a wide-meshed network in the coagulated blood. Hematoxylin and eosin. × 40.

Fig. 4. Higher magnification of detail from Fig. 3. Adjacent to the millipore filter (mf) a zone of fibroblasts and connective tissue fibres (f) is seen. Around the gelatin sponge several mononuclear cells. × 100.

Fig. 5. Section of chamber containing Spongostan + blood after intraperitoneal implantation for 3 weeks. The chamber is for the greater part filled with granulation tissue. Around small remnants of the sponge clusters of mononuclear cells and several foreign body giant cells are seen. The giant cell (g) in the middle of the picture has several nuclei and a diameter of some 80 microns. Goldner's Masson. × 250.

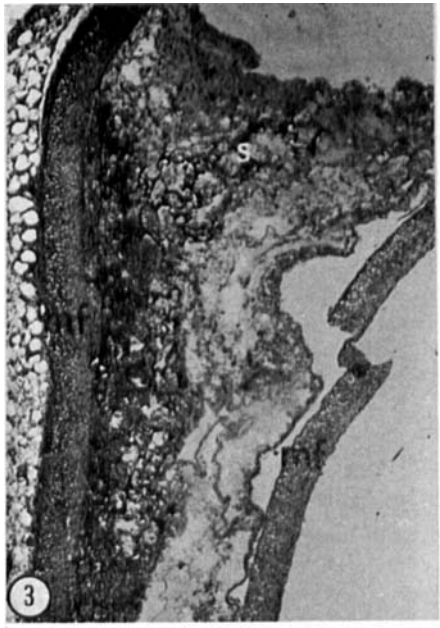
Fig. 6. Detail of section from chamber containing Spongostan + blood placed subcutaneously for 2 months. The chamber is filled with connective tissue rich in cells and capillaries. Rests of unresorbed sponge (s) are still present, surrounded by accumulations of lymphocytes (l) and macrophages (m). In the coloured preparations ingested particles within the cytoplasm of the macrophages are clearly visible. Goldner's Masson. × 400.

Fig. 7. Section from chamber containing Oxycel + blood after subcutan implantation for 1 week. Heavy accumulations of inflammatory cells, mainly lymphocytes (l) and plasma, cells (p), are seen in the pores of the implanted material (o). mf = millipore filter. Hematoxylin and eosin. × 100

Fig. 8. Section from chamber containing Oxycel + blood implanted subcutaneously for 2 months. Rests of unresorbed Oxycel (o) are surrounded by cell-rich granulation tissue. The predominant cells are macrophages (m) with the cytoplasm filled with ingested particles. Also several lymphocytes (l) and plasma cells (p) are seen. Goldner's Masson. × 250.

Fig. 9. Section from control chamber containing only blood, placed intraperitoneally for 1 week. Lots of invading cells are seen in the pores of the millipore filter (mf). The chamber contents consist of a blood coagulum in a state of organization. A narrow zone of fibroblasts and collagen fibres is seen adjacent to the filter side. In the more central part of the chamber macrophages and different types of leukocytes are seen scattered among the red blood cells. Goldner's Masson. × 250.

Fig. 10. Section from control chamber implanted intraperitoneally for 5 weeks. The blood coagulum is now substituted by connective tissue. Regularly arranged fibroblasts and collagen fibres are seen adjacent to the millipore filter. In the central part of the chamber the tissue is more rich in cells and capillaries (c). Goldner's Masson. × 250.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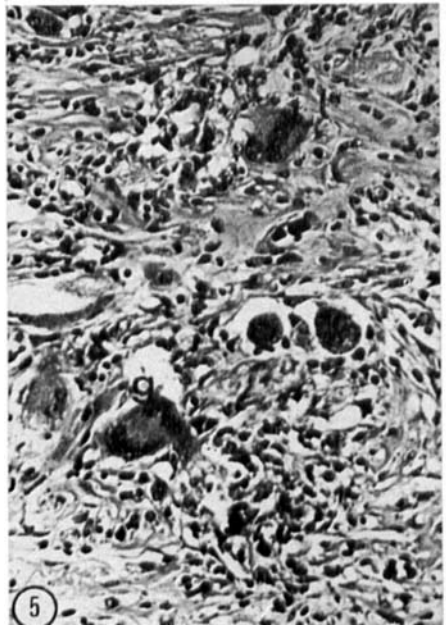
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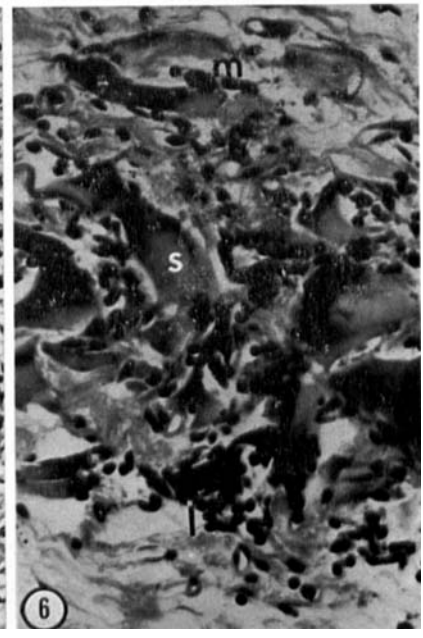
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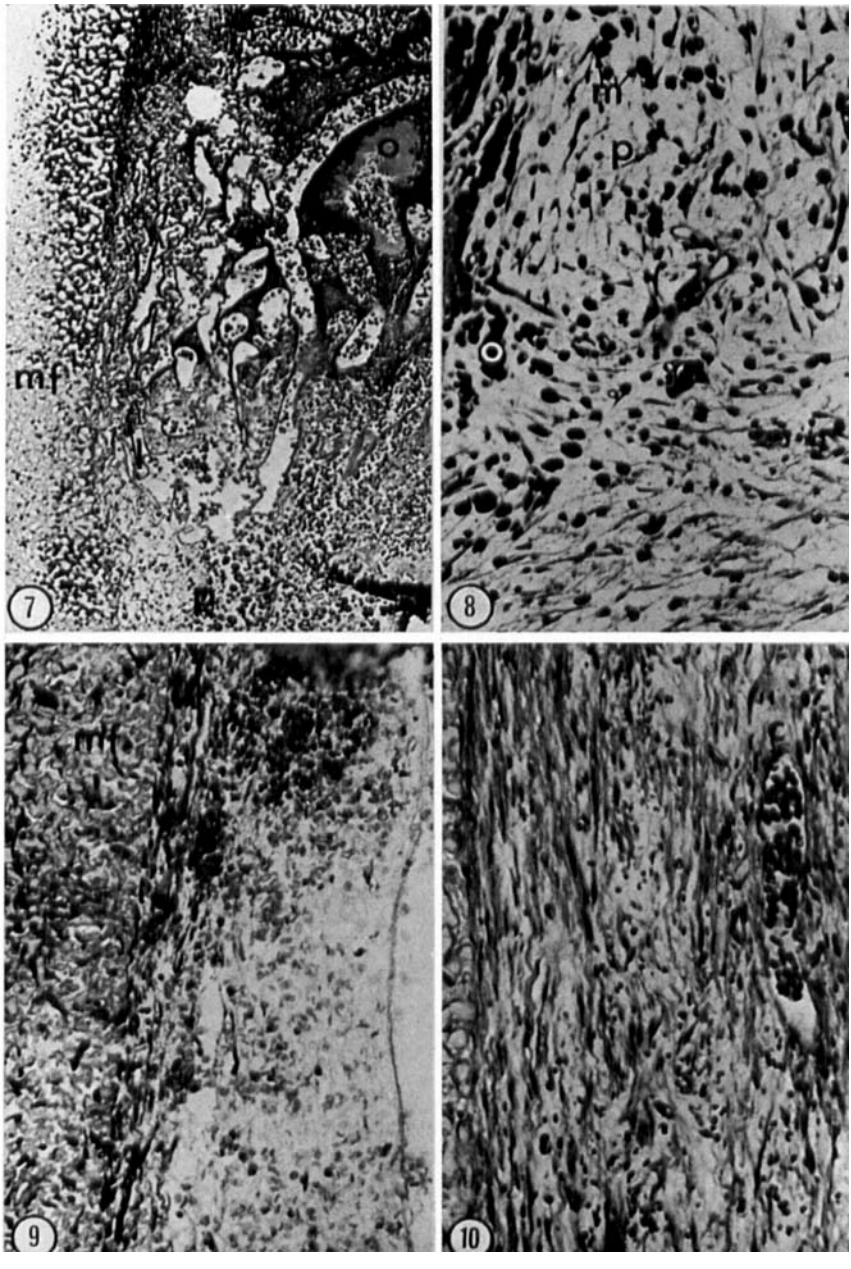
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there were still considerable amounts of unresorbed cellulose. The cellular reaction had subsided, but in the meshes of the implanted material cellular fragments and debris were seen.

Two months after implantation most of the implanted material had disappeared from the chambers. Unresorbed rests were surrounded by a cell-rich granulation tissue of which the macrophages were the predominant cells (Fig. 8).

Common for both Oxycel and Spongostan was that the ingrowth of new tissue seemed to progress slightly better in chambers where the implant was embedded in blood, than in chambers where it was moistened in physiologic salt solution. No significant difference was noticed as to resorption of the implanted material and ingrowth of connective tissue in chambers which were placed subcutaneously from those that were placed in the peritoneal cavity.

*Controls.* In chambers filled with only blood, there was after 1 week a blood clot in the state of organization (Fig. 9). The chamber was enveloped by connective tissue which infiltrates into the pores of the millipore filters. Adjacent to the chamber walls there was a border of fibroblasts and collagen fibres. More centrally in the clot, leukocytes, macrophages and fibroblasts were scattered among the red blood cells. After 5 weeks the organization process was ended, and the chamber was filled with connective tissue (Fig. 10).

## DISCUSSION

For Spongostan as well as Oxycel the histological examination revealed a strong cellular inflammatory reaction during the first 2 weeks after implantation. In the pores of the materials, large amounts of leukocytes and disintegrated cellular fragments were found. In the central parts of the implants, the cellular picture often resembled that of pus. After approximately 3 weeks, the cellular reaction decreased to some extent, but as far as Oxycel was concerned, there were still large amounts of leukocytes adjacent to the agent. The materials were gradually resorbed, and the chambers were eventually filled with connective tissue. The resorption process seemed to be rather slow, indicated by the fact that there were remnants of uncresorbed material after 2 months.

These results differ to some extent from those obtained in previous investigations in this field. The authors mentioned above (*Frantz*, 1943; *Burns*, 1946; *Bing*, 1947) found that oxidized cellulose as well as gelatin sponge caused but a mild tissue reaction and disappeared some 3—4 weeks after implantation in animal abdominal cavities. However, the methods employed in these investigations rendered it extremely difficult to localize small amounts

of the material 3--4 weeks after the implantation. The agent may then have been dislocated from the original site of implantation, and the non-resorbed remnants covered by connective tissue, to the effect that the macroscopic discovery of implanted residuals is hardly possible. By the insertion of gelatin after tooth extractions, as done by *Blomqvist et al.* (1962), the implanted material will tend to slip out from the socket, even if it is closed with sutures, a process which is often observed clinically.

Due to the technique used in the present investigation, the implanted agent was definitely fixed in its position, consequently offering no problems as to its localization at the time of removal for the subsequent histological examination. Cells as well as capillaries penetrated the filter pores, the experimental conditions thus differing very little from the direct inlay technique. Furthermore, the control chambers, containing blood only, show that the blood clot in the chamber was organized in the usual way (Figs. 9, 10).

*Bing* (1947) found that if the insertion of gelatin sponge was done without regard to sterility, there was primarily a strong leukocyte reaction. At the same time there seemed to be a fusion of the sponge into solid pieces, which underwent a slower resorption than when the sponge was implanted under sterile precautions. It is, however, virtually impossible to undertake any surgical operation under sterile conditions in the mounth. The microbial flora of the oral cavity will always, to some extent, directly or indirectly, have access to the wound.

*Lindstrom* (1956) sealed gelatin sponge in agar inoculated with Staph. albus, and cultivated under anaerobic conditions. He found abundant growth of the aerobic microbes around the sponge, while controls without the porous gelatin showed very little growth. He concluded from these findings that the air contents of the gelatin sponge may facilitate the invasion of aerobic micro-organisms which, in the average closed wound, would tend to die. Aerobic micrococci of the type Staph. aureus and Staph. albus are members of the normal flora of the skin and upper respiratory tract of man, and are acquired the first few days of life by contact and by airborne contamination. Consequently, wound contamination with these microbes is in most cases unavoidable.

It is difficult to state any definite opinion as to the role of infection with regard to the results obtained in the present investigation, as no attempts were made to demonstrate the presence of micro-organisms in the chambers. All working procedures were done as aseptically as possible. In what way infection could possibly have altered the physical properties of the materials, making them less resorbable, as indicated by *Bing* (1947), is not known.

As judged from the histological sections, the resorption of both agents seems mainly to be effected through phagocytosis. Numerous macrophages

with their cytoplasm laden with engulfed particles were found adjacent to the materials (Figs. 6, 8). In some cases such cells were also seen in the connective tissue just outside the chamber. These macrophages must then have emigrated through the millipore membranes with their contents of ingested material. Especially in the chambers containing Spongostan, numerous foreign body giant cells were seen. Many of these were of considerable size, with a diameter of from 40 to 80 microns (Fig. 5). Considering that the diameter of the chamber pores was approximately 14 microns, these cells could hardly have invaded the chamber from the surrounding tissues, but must originate from smaller cells inside the chamber. This may have occurred by fusion of several smaller cells, or by mitotic division of the nucleus without simultaneous division of the cytoplasm. The former view is to-day assumed to be the most likely explanation considering the origin of multinucleate giant cells (*Bloom & Fawcett*, 1968).

One is not allowed to draw direct conclusions from an animal experimental investigation as applicable to the conditions in man. However, the strong cellular reaction the first 2—3 weeks after implantation, seems to indicate the necessity of a certain caution in using resorbable hemostatic agents as gelatin sponge and oxidized cellulose, when these materials are implanted into wound cavities followed by primary closure. If the inflammatory reaction is due to the materials' ability to facilitate the invasion of aerobic microbes, this condition must necessarily be of the greatest importance when such agents are used during surgical procedures in the mouth, where the microbial flora of the oral cavity will unavoidably gain access to the operation field.

## SUMMARY

Gelatin sponge (Spongostan) and oxidized cellulose (Oxycel) were moistened with autogenous blood and placed in cell-permeable millipore chambers which were implanted intraperitoneally or subcutaneously in rats. The chambers were removed and investigated histologically at periods ranging from 1 week to 2 months. A total of 52 chambers in 26 animals were implanted.

The histological examination revealed a strong leukocytic reaction to the agents the first 2—3 weeks after the implantation. In some cases, especially in chambers containing Oxycel, the cellular picture had the morphological characteristics of pus. The cellular reaction gradually subsided, and the chambers were eventually filled with connective tissue. The resorption process seemed to be slow, in both preparations there were unresorbed rests left in the chambers after 2 months.

The role of infection in connection with resorbable implants is discussed. It is cautioned against uncritical use of resorbable hemostatic agents, especially when they are left in infected wound cavities which are closed by primary sutures.

## RÉSUMÉ

### LE SORT DES IMPLANTS HÉMOSTATIQUES RÉSORBABLES CHEZ LES RATS

Des implants intrapéritonéaux et sous-cutanés consistant en éponge de gélatine (Spongostan) et en cellulose oxydée (Oxycel) imbibées de sang autogène et placées dans des chambres millipores perméables aux cellules, ont été mis en place chez des rats. Au bout de périodes allant d'une semaine à 2 mois, les chambres ont été retirées et soumises à un examen histologique. Au total 52 chambres ont été implantées sur 26 animaux.

L'examen histologique a mis en évidence une forte réaction leucocytaire envers ces produits pendant les 2—3 premières semaines après l'implantation. Dans quelques cas, particulièrement dans les chambres contenant l'Oxycel, l'image cellulaire présentait les caractères morphologiques du pus. La réaction cellulaire diminuait progressivement, et les chambres finissaient par être remplies de tissu conjonctif. Le processus de résorption semblait lent; au bout de deux mois, il y avait dans les chambres des restes non résorbés des deux produits.

L'auteur discute le rôle de l'infection lors de l'emploi d'implants résorbables. Il recommande de ne pas employer sans discernement les agents hémostatiques résorbables, particulièrement lorsqu'ils sont laissés dans des cavités de plaies infectées fermées par sutures primaires.

## ZUSAMMENFASSUNG

### RESORBIERBARE HEMOSTATIKA BEI IMPLANTATION IN RATTEN

Gelatineschwamm (Spongostan) und oxidierte Zellulose (Oxycel) wurden mit autogenem Blut getränkt, und in zell- und kapillardurchlässige millipore-Kammern intraperitoneal oder subcutan in Ratten eingepflanzt. Nach Implantationsdauer von 1 Woche bis 2 Monaten wurden die Kammern entfernt und histologisch untersucht. Im Ganzen wurden 52 Kammern in 26 Versuchstiere implantiert.

Die histologische Untersuchung zeigte eine starke Leukozytenreaktion bei beiden Präparaten die ersten 2—3 Wochen nach der Implantation. In einigen Fällen, meistens in den Oxycelenthaltenden Kammern, kam es zu Eiter-

bildung. Die zelluläre Reaktion nahm langsam ab, und die Kammern wurden allmählich mit Bindegewebe gefüllt. Die Resorptionsprozess schien ganz langsam zu verlaufen. Histologisch konnte man noch nach 2 Monaten Reste von den implantierten Materialien in den Kammern finden.

Die Entstehung von Infektion in Zusammenhang mit dem Einlegen von resorbierbaren Materialien ist zur Diskussion gestellt. Es wird vor unkritischem Gebrauch sogenannter resorbierbarer Präparate gewarnt, besonders wenn sie in infizierten Wunden eingeschlossen werden.

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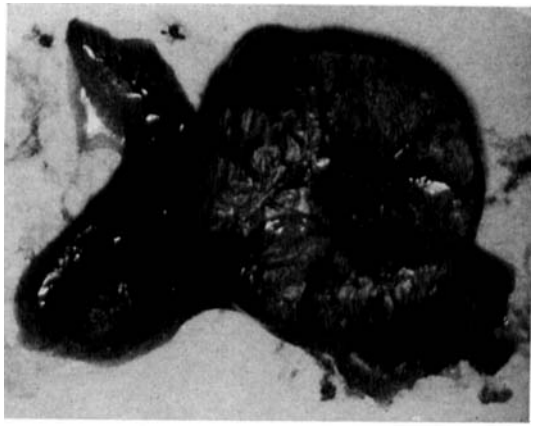


Fig. 2. Millipore-chamber 2 weeks after implantation in the peritoneal cavity. The chamber is enveloped by well vascularized connective tissue.

## RESULTS

At removal the chambers were surrounded by well vascularized connective tissue, which strongly adhered to the filter membrans (Fig. 2). The formation of pus around the chambers was never observed. In 3 cases some serous exudate was found adjacant to the chambers.

### *Microscopic observations*

*Chambers filled with Spongostan and blood.* The contents of the 1-week-old chambers consisted of meshes and strands of the gelatin sponge, embedded in coagulated blood (Fig. 3). Collections of leukocytes were seen surrounding the implanted material. Adjacent to the filter side there was a border of fibroblasts and collagen fibres (Fig. 4). Two weeks after implantation heavy accumulations of mononuclear cells were seen in the pores of the implanted material. Three weeks after implantation a great proportion of the implanted material had disappeared from the chambers (Fig. 5). The leukocyte reaction had subsided; around remnants of unreasorbed gelatin, lymphocytes and macrophages as well as many foreign body giant cells were seen. In the 4 and 5-week-old chambers there was a gradual diminishing of the implanted sponge. However, in some of the chambers considerable amounts were still left. Unresorbed rests were surrounded by cell-rich granulation tissue. Two months after implantation the chambers were for the greater part filled with connective tissue in which several capillaries were seen. There were still remnants of unresorbed sponge which were surrounded by macrophages and mononuclear cells (Fig. 6).

*Chambers filled with Oxycel and blood.* One week after implantation there was a very strong cellular reaction to the implanted material, with heavy accumulations of leukocytes and plasma cells (Fig. 7). After 2 weeks, ingrowth of connective tissue with many capillaries was seen adjacent to the filter side. In the central part of the chambers there were abundant inflammatory cells with degenerative changes. In several areas the cellular picture had the morphological characteristics of pus.

Three weeks after implantation there was still a strong cellular reaction and accumulations of disintegrated cells adjacent to the implanted material, which, in some instances, seemed to have condensed into solid masses. In the 4 and 5-week-old chambers the ingrowth of connective tissue was progressing, but

Fig. 3. Section of chamber containing Spongostan + blood placed intraperitoneally for 1 week. The implanted gelatin sponge (s) is seen between the millipore filters (mf) having the appearance of a wide-meshed network in the coagulated blood. Hematoxylin and eosin. × 40.

Fig. 4. Higher magnification of detail from Fig. 3. Adjacent to the millipore filter (mf) a zone of fibroblasts and connective tissue fibres (f) is seen. Around the gelatin sponge several mononuclear cells. × 100.

Fig. 5. Section of chamber containing Spongostan + blood after intraperitoneal implantation for 3 weeks. The chamber is for the greater part filled with granulation tissue. Around small remnants of the sponge clusters of mononuclear cells and several foreign body giant cells are seen. The giant cell (g) in the middle of the picture has several nuclei and a diameter of some 80 microns. Goldner's Masson. × 250.

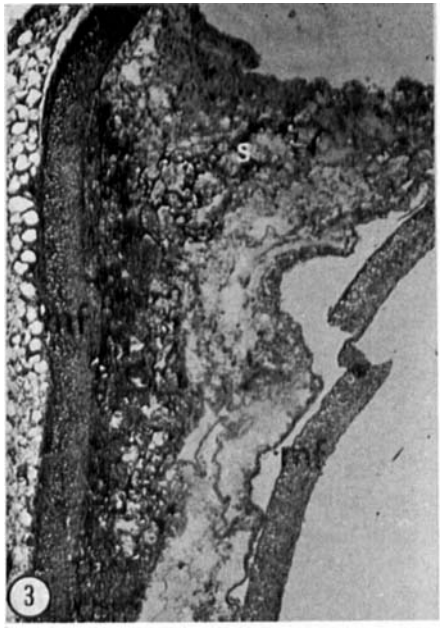
Fig. 6. Detail of section from chamber containing Spongostan + blood placed subcutaneously for 2 months. The chamber is filled with connective tissue rich in cells and capillaries. Rests of unresorbed sponge (s) are still present, surrounded by accumulations of lymphocytes (l) and macrophages (m). In the coloured preparations ingested particles within the cytoplasm of the macrophages are clearly visible. Goldner's Masson. × 400.

Fig. 7. Section from chamber containing Oxycel + blood after subcutan implantation for 1 week. Heavy accumulations of inflammatory cells, mainly lymphocytes (l) and plasma, cells (p), are seen in the pores of the implanted material (o). mf = millipore filter. Hematoxylin and eosin. × 100

Fig. 8. Section from chamber containing Oxycel + blood implanted subcutaneously for 2 months. Rests of unresorbed Oxycel (o) are surrounded by cell-rich granulation tissue. The predominant cells are macrophages (m) with the cytoplasm filled with ingested particles. Also several lymphocytes (l) and plasma cells (p) are seen. Goldner's Masson. × 250.

Fig. 9. Section from control chamber containing only blood, placed intraperitoneally for 1 week. Lots of invading cells are seen in the pores of the millipore filter (mf). The chamber contents consist of a blood coagulum in a state of organization. A narrow zone of fibroblasts and collagen fibres is seen adjacent to the filter side. In the more central part of the chamber macrophages and different types of leukocytes are seen scattered among the red blood cells. Goldner's Masson. × 250.

Fig. 10. Section from control chamber implanted intraperitoneally for 5 weeks. The blood coagulum is now substituted by connective tissue. Regularly arranged fibroblasts and collagen fibres are seen adjacent to the millipore filter. In the central part of the chamber the tissue is more rich in cells and capillaries (c). Goldner's Masson. × 250.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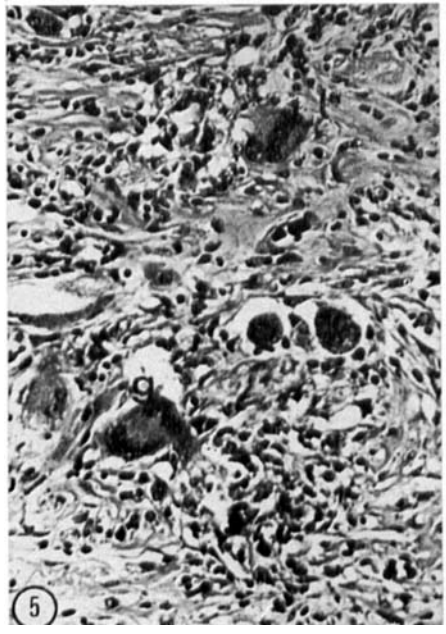
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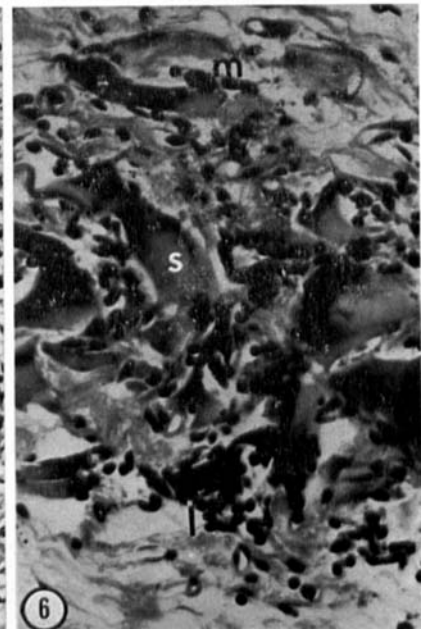
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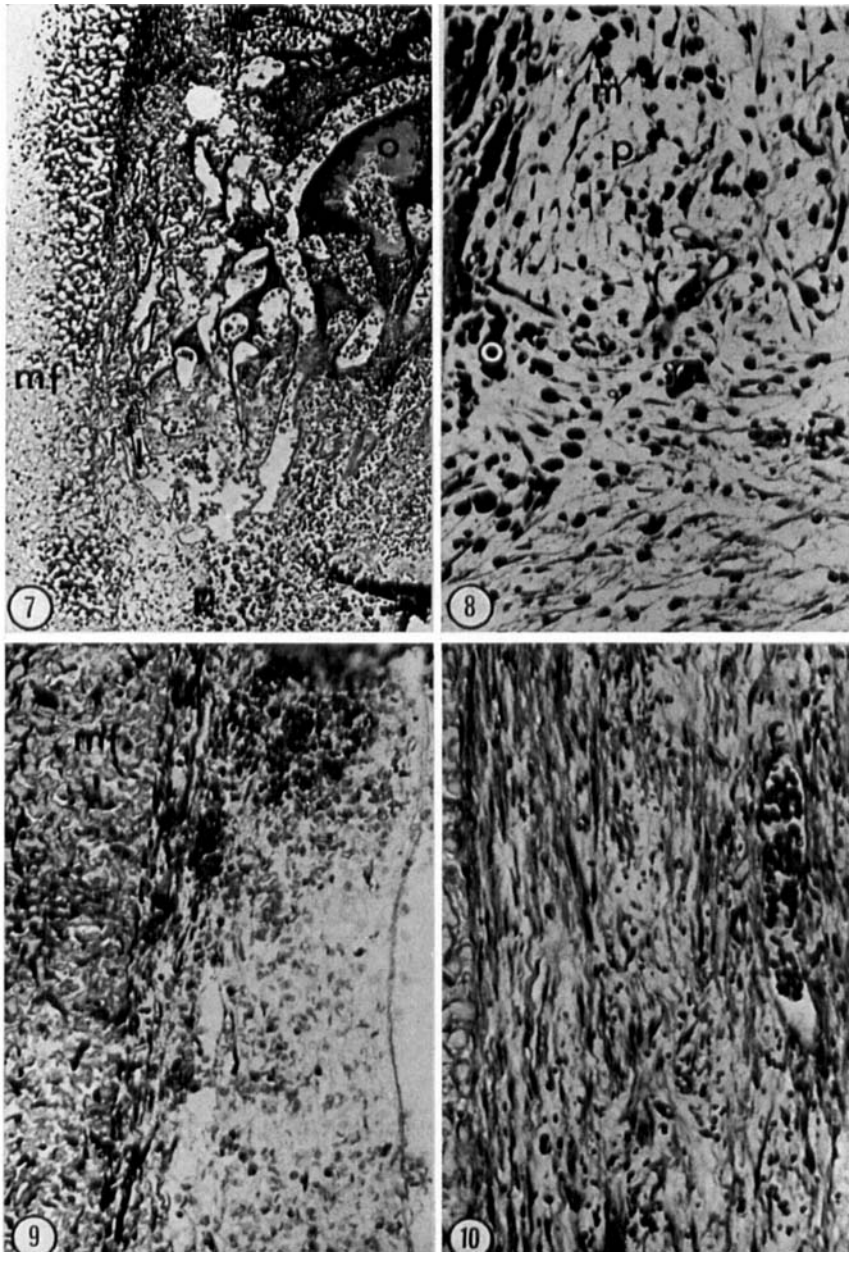
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there were still considerable amounts of unresorbed cellulose. The cellular reaction had subsided, but in the meshes of the implanted material cellular fragments and debris were seen.

Two months after implantation most of the implanted material had disappeared from the chambers. Unresorbed rests were surrounded by a cell-rich granulation tissue of which the macrophages were the predominant cells (Fig. 8).

Common for both Oxycel and Spongostan was that the ingrowth of new tissue seemed to progress slightly better in chambers where the implant was embedded in blood, than in chambers where it was moistened in physiologic salt solution. No significant difference was noticed as to resorption of the implanted material and ingrowth of connective tissue in chambers which were placed subcutaneously from those that were placed in the peritoneal cavity.

*Controls.* In chambers filled with only blood, there was after 1 week a blood clot in the state of organization (Fig. 9). The chamber was enveloped by connective tissue which infiltrates into the pores of the millipore filters. Adjacent to the chamber walls there was a border of fibroblasts and collagen fibres. More centrally in the clot, leukocytes, macrophages and fibroblasts were scattered among the red blood cells. After 5 weeks the organization process was ended, and the chamber was filled with connective tissue (Fig. 10).

## DISCUSSION

For Spongostan as well as Oxycel the histological examination revealed a strong cellular inflammatory reaction during the first 2 weeks after implantation. In the pores of the materials, large amounts of leukocytes and disintegrated cellular fragments were found. In the central parts of the implants, the cellular picture often resembled that of pus. After approximately 3 weeks, the cellular reaction decreased to some extent, but as far as Oxycel was concerned, there were still large amounts of leukocytes adjacent to the agent. The materials were gradually resorbed, and the chambers were eventually filled with connective tissue. The resorption process seemed to be rather slow, indicated by the fact that there were remnants of uncresorbed material after 2 months.

These results differ to some extent from those obtained in previous investigations in this field. The authors mentioned above (*Frantz*, 1943; *Burns*, 1946; *Bing*, 1947) found that oxidized cellulose as well as gelatin sponge caused but a mild tissue reaction and disappeared some 3—4 weeks after implantation in animal abdominal cavities. However, the methods employed in these investigations rendered it extremely difficult to localize small amounts

of the material 3--4 weeks after the implantation. The agent may then have been dislocated from the original site of implantation, and the non-resorbed remnants covered by connective tissue, to the effect that the macroscopic discovery of implanted residuals is hardly possible. By the insertion of gelatin after tooth extractions, as done by *Blomqvist et al.* (1962), the implanted material will tend to slip out from the socket, even if it is closed with sutures, a process which is often observed clinically.

Due to the technique used in the present investigation, the implanted agent was definitely fixed in its position, consequently offering no problems as to its localization at the time of removal for the subsequent histological examination. Cells as well as capillaries penetrated the filter pores, the experimental conditions thus differing very little from the direct inlay technique. Furthermore, the control chambers, containing blood only, show that the blood clot in the chamber was organized in the usual way (Figs. 9, 10).

*Bing* (1947) found that if the insertion of gelatin sponge was done without regard to sterility, there was primarily a strong leukocyte reaction. At the same time there seemed to be a fusion of the sponge into solid pieces, which underwent a slower resorption than when the sponge was implanted under sterile precautions. It is, however, virtually impossible to undertake any surgical operation under sterile conditions in the mounth. The microbial flora of the oral cavity will always, to some extent, directly or indirectly, have access to the wound.

*Lindstrom* (1956) sealed gelatin sponge in agar inoculated with Staph. albus, and cultivated under anaerobic conditions. He found abundant growth of the aerobic microbes around the sponge, while controls without the porous gelatin showed very little growth. He concluded from these findings that the air contents of the gelatin sponge may facilitate the invasion of aerobic micro-organisms which, in the average closed wound, would tend to die. Aerobic micrococci of the type Staph. aureus and Staph. albus are members of the normal flora of the skin and upper respiratory tract of man, and are acquired the first few days of life by contact and by airborne contamination. Consequently, wound contamination with these microbes is in most cases unavoidable.

It is difficult to state any definite opinion as to the role of infection with regard to the results obtained in the present investigation, as no attempts were made to demonstrate the presence of micro-organisms in the chambers. All working procedures were done as aseptically as possible. In what way infection could possibly have altered the physical properties of the materials, making them less resorbable, as indicated by *Bing* (1947), is not known.

As judged from the histological sections, the resorption of both agents seems mainly to be effected through phagocytosis. Numerous macrophages

with their cytoplasm laden with engulfed particles were found adjacent to the materials (Figs. 6, 8). In some cases such cells were also seen in the connective tissue just outside the chamber. These macrophages must then have emigrated through the millipore membranes with their contents of ingested material. Especially in the chambers containing Spongostan, numerous foreign body giant cells were seen. Many of these were of considerable size, with a diameter of from 40 to 80 microns (Fig. 5). Considering that the diameter of the chamber pores was approximately 14 microns, these cells could hardly have invaded the chamber from the surrounding tissues, but must originate from smaller cells inside the chamber. This may have occurred by fusion of several smaller cells, or by mitotic division of the nucleus without simultaneous division of the cytoplasm. The former view is to-day assumed to be the most likely explanation considering the origin of multinucleate giant cells (*Bloom & Fawcett*, 1968).

One is not allowed to draw direct conclusions from an animal experimental investigation as applicable to the conditions in man. However, the strong cellular reaction the first 2—3 weeks after implantation, seems to indicate the necessity of a certain caution in using resorbable hemostatic agents as gelatin sponge and oxidized cellulose, when these materials are implanted into wound cavities followed by primary closure. If the inflammatory reaction is due to the materials' ability to facilitate the invasion of aerobic microbes, this condition must necessarily be of the greatest importance when such agents are used during surgical procedures in the mouth, where the microbial flora of the oral cavity will unavoidably gain access to the operation field.

## SUMMARY

Gelatin sponge (Spongostan) and oxidized cellulose (Oxycel) were moistened with autogenous blood and placed in cell-permeable millipore chambers which were implanted intraperitoneally or subcutaneously in rats. The chambers were removed and investigated histologically at periods ranging from 1 week to 2 months. A total of 52 chambers in 26 animals were implanted.

The histological examination revealed a strong leukocytic reaction to the agents the first 2—3 weeks after the implantation. In some cases, especially in chambers containing Oxycel, the cellular picture had the morphological characteristics of pus. The cellular reaction gradually subsided, and the chambers were eventually filled with connective tissue. The resorption process seemed to be slow, in both preparations there were unresorbed rests left in the chambers after 2 months.

The role of infection in connection with resorbable implants is discussed. It is cautioned against uncritical use of resorbable hemostatic agents, especially when they are left in infected wound cavities which are closed by primary sutures.

## RÉSUMÉ

### LE SORT DES IMPLANTS HÉMOSTATIQUES RÉSORBABLES CHEZ LES RATS

Des implants intrapéritonéaux et sous-cutanés consistant en éponge de gélatine (Spongostan) et en cellulose oxydée (Oxycel) imbibées de sang autogène et placées dans des chambres millipores perméables aux cellules, ont été mis en place chez des rats. Au bout de périodes allant d'une semaine à 2 mois, les chambres ont été retirées et soumises à un examen histologique. Au total 52 chambres ont été implantées sur 26 animaux.

L'examen histologique a mis en évidence une forte réaction leucocytaire envers ces produits pendant les 2—3 premières semaines après l'implantation. Dans quelques cas, particulièrement dans les chambres contenant l'Oxycel, l'image cellulaire présentait les caractères morphologiques du pus. La réaction cellulaire diminuait progressivement, et les chambres finissaient par être remplies de tissu conjonctif. Le processus de résorption semblait lent; au bout de deux mois, il y avait dans les chambres des restes non résorbés des deux produits.

L'auteur discute le rôle de l'infection lors de l'emploi d'implants résorbables. Il recommande de ne pas employer sans discernement les agents hémostatiques résorbables, particulièrement lorsqu'ils sont laissés dans des cavités de plaies infectées fermées par sutures primaires.

## ZUSAMMENFASSUNG

### RESORBIERBARE HEMOSTATIKA BEI IMPLANTATION IN RATTEN

Gelatineschwamm (Spongostan) und oxidierte Zellulose (Oxycel) wurden mit autogenem Blut getränkt, und in zell- und kapillardurchlässige millipore-Kammern intraperitoneal oder subcutan in Ratten eingepflanzt. Nach Implantationsdauer von 1 Woche bis 2 Monaten wurden die Kammern entfernt und histologisch untersucht. Im Ganzen wurden 52 Kammern in 26 Versuchstiere implantiert.

Die histologische Untersuchung zeigte eine starke Leukozytenreaktion bei beiden Präparaten die ersten 2—3 Wochen nach der Implantation. In einigen Fällen, meistens in den Oxycelenthaltenden Kammern, kam es zu Eiter-

bildung. Die zelluläre Reaktion nahm langsam ab, und die Kammern wurden allmählich mit Bindegewebe gefüllt. Die Resorptionsprozess schien ganz langsam zu verlaufen. Histologisch konnte man noch nach 2 Monaten Reste von den implantierten Materialien in den Kammern finden.

Die Entstehung von Infektion in Zusammenhang mit dem Einlegen von resorbierbaren Materialien ist zur Diskussion gestellt. Es wird vor unkritischem Gebrauch sogenannter resorbierbarer Präparate gewarnt, besonders wenn sie in infizierten Wunden eingeschlossen werden.

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