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Histological Investigations on the Healing Process of Vascular Prostheses

Badania histologiczne nad procesem wgajania się protez naczyniowych

Гистологические исследования над вживлением сосудистых протезов

The application of vascular grafts and prostheses contributed to a rapid development of blood vessel surgery. Difficulties, complications, and failures associated with the application of autogenous, homogenous, and heterologous grafts necessitated the search for other substitutive materials. Attempts to use tubes of nonbiological materials were undertaken a long time ago. Carrel (4) inserted paraffined tubes of glass or aluminium into the aorta of the dog. Tuffier (quot. after 6) used similar tubes of silver. Tubes of ivory, rubber, and other materials were also used. These attempts, however, resulted in failure. Only the use of synthetic material — Vinyon-N—as vascular prostheses by Voorhees, Jaretzki, and Blakemore in 1952 (23) proved to be a real improvement. Since that time numerous experimental studies and clinical observations on several synthetic materials have been reported (1—3, 7, 8, 11, 14, 16—19, 22). Nylon, orlon, dacron, teflon, vinyon-N, ivalon, terylen were studied most frequently (9, 10, 12, 13, 15, 20). An essential factor in the application of prostheses of synthetic material is their healing process and the formation of a substitutive vessel in the place of a defect.

MATERIAL AND METHODS

Synthetic vascular prostheses made of terital * were used in the experiments. Terital (...OCH_2CH_2O—OCC_6H_4CO...) is polyester obtained from terephtalic acid and glycol. The experimental studies were carried out on 10 healthy dogs of mixed breed, weighing from 15 to 34 kg, their age ranging from 1—4 years.

* The prostheses were made in the Central Laboratory of the Textile Industry in Łódź.

After anaesthetizing the animals by tubation narcosis the abdomen was entered through a midline incision, and the aorta was approached and exposed from the level of the renal vessels to the trifurcation. Segments of the aorta from 4 to 5 cm were then removed from between two vascular clamps. Next, the continuity of the vessel was restored by segments of the prosthesis, 5 to 6 cm long, sterilized by boiling, the diameter of which was a little larger than that of the dog's aorta. The abdominal cavity was closed then. After the observation period lasting from 12 hours to 272 days, the dogs were sacrificed, and the prosthesis was cut out together with the adhering ends of the aorta for macroscopic evaluation and histological studies. The material for histological studies was taken from the region of the junctions and from the midgraft area. It was fixed in Schaffen's liquid (alcohol — formol) for 24 hours, dehydrated through alcohols, cleared in xylon, and embedded in paraffine. Microtome section (10—15 μ) after deparaffinizing were stained with haematoxylin and eosin, with a triple stain after Masson, and with resorcine-fuxin after Weigert.

RESULTS

Twelve hours after the operation the following microscopic picture was obtained: the inner surface of the prosthesis was covered with a layer of fibrine forming a net of fine meshes which were abundantly filled with morphologic blood elements, so that in several places it appeared as parietal thrombosis. The layer filled up the depressions resulting from the folding of the prosthesis. Also the angle formed by outward backfolding of the edges of the aorta and the prosthesis in the place of their junction was filled with fibrine saturated with morphologic blood elements. This layer passed on through the junction to the wall of the aorta. The prosthesis pores as well as the interstices of terital were filled with fibrine and morphologic blood elements. The outer surface of the prosthesis was also covered with a fibrine layer containing morphologic blood elements, a great number of which resembled thrombosis.

After 7 days of observation it was noticed that the fibrine membrane of the inner surface was formed of two apparent layers: one consisting of a considerably thick fibrinous underlying, which surrounded closely every fibre and yarn of the prosthesis containing morphologic blood elements. On this layer, from the side of the lumen of the graft, there was found a parietal thrombosis (Fig. 1) with a big concentration of mononuclear elements (leukocytes, monocytes, lymphocytes). Neither cells nor fibres of the connective tissue were observed in this layer except in the places of junction, where single stripes of connective tissue passed on from the aorta to the prosthesis on the surface of the fibrous layer. In the interstices between particular fibres of terital and in the pores of the prosthesis an abundant concentration with

few morphological blood elements was visible. The outer surface of the prosthesis, however, was covered with an organizing fibrinous membrane in which blood cells and phagocytes of the reticulo-endothelial system were observed.

After 14 days round cells, fibroblasts, fibrocytes, and histiocytes were visible within the fibrous mass on the inner surface of the prosthesis. Characteristic was the regular arrangement of fibrocytes close to the inner surface. In some places, however, the fibrinous layer was not penetrated by the above mentioned elements of connective tissue and did not show any features of organization. Within the prosthesis itself closely clustered fibrocytes were visible in the fibrinous mass. On the outer surface of the prosthesis distinct features of organization in the form of hypertrophy of the connective tissue elements were found.

After 21 days in some parts of the prosthesis, which was not surrounded by a haematoma but in touch with the neighbouring tissue, growth of connective tissue elements between yarns and fibres of terital could be observed. Cells which by their appearance resembled fibroblasts chiefly filled wide spaces between yarns of the material and their thin projects, and tissue fibres arranged themselves along and round single fibres of terital causing a tightening of the prosthesis (Fig. 2). In the peripheral parts of the prosthesis wall larger spaces were filled above all with cells and their projects, and fibres of connective tissue. In this case single fibres of terital were drawn into the basal layer and strongly associated with it. The inner layer lining the prosthesis near the junction was composed of well developed elements of connective tissue. In the central part of the prosthesis there were visible single cells and fibres of connective tissue and unorganized fibrine in the net of which blood cells were present. In other places typical parietal thrombosis occurred being connected with the thrombosis which occupied the whole lumen of the prosthesis. In the prosthesis wall neighbouring with the undeveloped part of the inner layer a very small number of connective tissue cells in the form of thin single fibres was observed, which filled the interstices of terital fibres. In wider spaces, particularly in cross sections glistening masses with remnants of blood fibrine could be noticed. In the connective tissue surrounding the prosthesis there was observed a formation of precapillary blood vessels with undifferentiated epithelium, which lined the prosthesis wall.

In dog No. 7 after 23 days there appeared a distinct difference in the inner layer near the junction and in the midgraft region. In the

region of the junction a dozen of connective tissue stripes covered with regular endothelium, passed on from the aorta to the prosthesis. The farther from the junction the more covered was the inner connective tissue layer with a slightly changed cubical epithelium. In some places it was covered with parietal thrombosis (Fig. 3). At the distance of 6 to 7 mm from the junction the inner layer was thinner and the stripes of connective tissue were delicate. In this segment the inner layer was slightly associated with the prosthesis and its ingrowth through the interstices of the material was very small. There were also unorganized foci. In the central segment of the prosthesis the inner layer was formed by a narrow stripe of connective tissue which adhered directly to it. From the side of the vessel lumen this stripe was covered in some places with parietal thrombosis with an irregular edge. On the inner surface of the thrombosis an epithelium was growing (Fig. 4). The outer layer was formed of connective tissue or of an organizing haematoma. The interstices between the prosthesis fibres were scarcely filled with morphogenic blood elements and fibrine, whereas in air spaces between the prosthesis yarns thick stripes of undifferentiated connective tissue with prevalent cell elements and a small number of fibres were noticed.

After 28 days in dog No. 8 a difference in the inner layer between its central part and the regions neighbouring with the junction was also observed. At the junctions on the basal fibrinous layer there were connective tissue stripes among which many phagocytes were present. From the side of the vessel lumen this layer was covered with squamous epithelium. In the midgraft there were fewer stripes of connective tissue which penetrated the fibrous scaffolding. Between particular laminae blood cells and fibrine were visible. Phagocytes were few. The outer layer in places with no perigraft haematoma, was very thick and built of connective tissue. Single multinucleated leukocytes were visible in it.

After 44 days there appeared a well formed outer layer of connective tissue elements and a considerably thinner (about 4 times) inner layer. These layers were joined by the connective tissue growing through the graft walls between two bundles of fibres, i. e., in places in which porous spaces of graft existed (Fig. 5, 6). Precapillary and capillary blood vessels grew in together with connective tissue (Fig. 6). The inner layer was totally built of fibrinous connective tissue with the prevalence of mature elements, i. e., fibrocytes and collagenic fibres. Cells and fibre bundles of connective tissue arranged themselves circularly most frequently. The outer part of this layer was closely

associated with terital fibres growing in between them and coating them. In this part of the layer single and also circularly running capillary blood vessels were observed. Nearer the vessel lumen, however, the inner layer did not contain capillary vessels, but only narrow fissures in which no blood cells were visible. The inner surface of this layer was formed of one stratum of connective tissue cells under which decomposing cells occurred which probably belonged to an earlier formed pariental thrombosis. In the place in which there was haematoma outside the prosthesis the inner layer was formed of a very thin connective tissue stripe under which decomposing blood elements and fibrine deposits were present (Fig. 13). In the prosthesis lumen fresh thrombosis was found which was not associated with the inner layer.

After 97 days the inner layer was formed of fibrinous connective tissue covered with epithelium from the inside. It was very closely associated with the prosthesis wall and grew in between the particular elements. In pores of the prosthesis also bundles of collagenic fibres and a small amount of connective tissue were present (Fig. 7). The outer layer, much more thicker than the inner one, was formed of connective tissue lamellae with the prevalence of fibrous elements. In this layer blood vessels were found growing in with stripes of connective tissue through the porous species of the prosthesis reaching the basal part of the inner layer.

After 201 days the inner layer at the junction and its neighbouring part was thick (above 1 mm). It was formed of flaccid connective tissue with a very small number of cell elements (Fig. 18), and covered with epithelium which was joined and was probably a continuation of the aorta endothelium. The farther from the junction the thinner was the inner layer; it was covered with a distinct visible pavement epithelium with undiscernable intercellular boundaries, which could prove its being undifferentiated (Fig. 9). The inner layer was well-associated with the prosthesis and penetrated between particular fibres and pores of the prosthesis. In free interstices of the prosthesis there was also a considerable amount of connective tissue with the prevalence of fibrous elements twisting strongly around terital fibres. In the central part around which a haematoma was present the inner layer was distinct and limited only to some connective tissue stripes with a great number of cell elements. This layer was covered with epithelium of a pavement character with indiscernible intercellular boundaries. Outside the prosthesis in places of its concretions with the environment a thick layer of connective tissue was found which grew closely

together with the prosthesis and was joined through pores with the inner layer. A part of the prosthesis surrounded with a haematoma did not possess such a layer.

After 272 days the inner connective tissue layer, in which, beside the proper fibres bundles of folded elastic fibres occurred (Fig. 10), was covered with epithelium. The epithelium showed a considerable growth in some places and sometimes it formed even some strata. The outer layer was also built of fibrous connective tissue with the prevalence of fibrinous elements; between them there were blood vessels, islands of lymphoid cells, and a large concentration of haemosiderine, which might prove the existence of a perigraft haematoma, which underwent an organization. The prosthesis wall was closely associated both with the external, considerably thicker layer and the internal thinner one. Those layers were joined by connective tissue stripes passing through the prosthesis.

DISCUSSION

On the basis of the results obtained the conclusion could be drawn that the implanted prosthesis segment in the defect of abdominal aorta reconstructing immediately the continuity of the vessel lumen, allows at once to restore a good functioning of the blood vessel, thanks to which no symptoms of disturbances in the hind legs of the experimental dogs were observed. The implanted prosthesis constituted an indispensable scaffolding on which the reconstruction of an excised segment of the abdominal aorta of the dog from the host's tissues took place. At first the blood flowing through the lumen of the prosthesis without any obstacle escaped in various quantities through the interstices of the graft which were then quite rapidly occluded with blood fibrine. Thus was accomplished the tightening of the prosthesis walls.

During the initial phase of the experiments in the place of prosthesis implantation three layers attracted attention in the microscopic picture: 1. the inner fibrinous layer, 2. the prosthesis itself being permeated by fibrine, 3. the outer fibrinous layer. The formation of these layers from the fibrine net which at the same time contained morphologic blood elements was a steady phenomenon. During the first days blood cells were penetrating into the inner layer.

The next stage of the prosthesis healing began at the moment in which there occurred organization of fibrinous layers by connective tissue elements which came from the ends of the host's vessel and the tissues surrounding the prosthesis. Of the two sources the surrounding

of prosthesis is very important. This importance was indicated by those cases in which the existence of a haematoma around the prosthesis hampered the ingrowth of connective tissue from the environment and delayed the organization of the fibrinous layers, particularly the inner one, not allowing the formation of a regular inner layer from connective tissue. This proves that the permeation of connective tissue from the vessel ends is not sufficient for a regular process of the prosthesis healing, and it indicates that a more important source of this process is the connective tissue surrounding the prosthesis. In all cases the organization of the inner layer was quicker in the neighbourhood of the junctions as compared with the central part of the graft being in these places always thicker. Moreover it was also covered with a monolayer stratum epithelium resembling endothelium of the aorta. The microscopic picture obtained proves that there exists unquestionable participation of the aorta in the organization of the inner layer. The growth of connective tissue into the fibrinous layer occurred some days after the operation. Seven days after the implantation only single stripes of connective tissue in the places of junction running from the aorta were observed. In the remaining parts of the inner layer no elements of connective tissue were found. These elements were also lacking in free interstices of the prosthesis which contained only fibrine and blood cells. The fibrinous outer layer, however, was in the initial phase of organization and it contained only histiocytes and phagocytes.

In the further course of the process a growth of connective tissue in all the three fibrine layers followed. The most distinct development of this process was noticed during the second week. After 14 days the presence of round cells, fibroblasts, fibrocytes, histiocytes in all layers was found, though not organized fields of fibrine could be met yet. At that time on the surface of the inner layer there was observed a regular arrangement of fibrocytes, which, morphologically, resembled the arrangement of epithelium cells.

The unfinished organization process of the inner fibrinous layer was still observed after 28 days, but after 44 days typical foci of unorganized fibrine were not found. These pictures do not show yet the completion of the prosthesis healing process, for even after 272 days on the surface of the inner connective tissue layer, besides the typical monolayer stratum epithelium, there occurred in some places epithelium composed of several layers of undifferentiated character which pointed to an active healing process of the prosthesis. Besides, in the later period there followed further transformation of the fibrinous tissue towards a typical fibrous one with a smaller number of cells.

In the second experimental period a three-layer stratification in the places of implantation could also be observed, namely: 1. inner connective tissue layer covered either with typical stratum epithelium or a pavement one, or a differentiated multilayer epithelium, or without any epithelium, 2. midlayer built of elements of connective tissue being at various differentiation stages and growing through the prosthesis wall and of the prosthesis itself, 3. outer connective tissue layer always considerably thicker than the inner one and being in the phase of a more advanced organization. Among the connective tissue elements in all the three layers blood vessels were found which grew in from inside through the interstices of the prosthesis reaching the inner layer. The presence of capillaries in the vessel walls throws some light on the process of nutrition of tissues forming these walls.

No cells of muscular tissue were found at all. P e t r y and H e b e r e r stated, however, that in the healing process of the prosthesis there occurs in its neighbourhood a transformation of fibrocytes into cells similar to those of smooth muscles. This transformation may be a reflexion of a functional adaptation of connective tissue.

The healing process of the vascular prosthesis of terital has nothing to do with the development of a typical vessel wall which contains all its elements. Above all there is a lack of muscular tissue and other constituents entering into the structure of the vascular wall. Thus, from the point of hemodynamics, this wall cannot be considered adequate, for being deprived of a sufficiently strong scaffolding in the form of a prosthesis it would undergo dilatation due to blood pressure or bursting. The prosthesis itself being built in the new-formed wall plays then a very important part in the preservation of its strength in a biological environment long time after implantation. In the investigations carried out neither a bursting of the wall occurred nor a haematoma was found; that proved the sufficient strength of the vascular prosthesis made of terital.

The investigations also showed that the healing process of the prosthesis did not develop regularly in its particular parts and it was usually quicker around the junctions as compared with the middle part. Moreover, this process did not develop parallel with the lapse of time. Many a time the healing process of the prosthesis was more advanced during a shorter period of observation that during a longer one. The formation of a haematoma round the prosthesis or even its part can undoubtedly exert a hampering influence on the healing process. The presence of haematoma prevents rapid growth of connective tissue elements from the neighbourhood into the fibrinous

layers which are formed immediately after the implantation of the prosthesis. The formation of a haematoma around the prosthesis is associated with too big a porosity of the prosthesis walls. In the material used haematomae occurred quite often (dogs 1, 2, 4 and 8). When considering the differences in the periods of the prosthesis healings biological characteristics of the animal and their influence on the healing process also had to be taken into account.

A separate discussion is needed for the occurrence of parietal thrombosis on the surface of the inner layer being already formed and covered with epithelium. These thrombi, as was proved, underwent another organization and covered themselves with epithelium. The lack of blood vessels in the inner layer, and strictly speaking, in its surface part to which epithelium adhered, may become the reason for undernourishing of the productive cells, and, as a consequence of degeneration and defects in epithelium itself, may directly lead to the formation of parietal thrombosis.

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FIGURES

Fig. 1. Inner layer formed from fibrine net strongly saturated with morphologic blood elements with a large concentration of rods, monocytes and lymphocytes. 7 days after operation. Stained after Masson. 400 ×.

Fig. 2. Ingrowth of connective tissue elements from outside between particular terital fibres. 21 days after operation. Stained with haematoxylin and eosin. 400 ×.

Fig. 3. Midgraft inner layer covered with an almost cubic epithelium with visible wall thrombosis adhering to the epithelium from the side of the prosthesis lumen. 23 days after operation. Masson staining. 190 ×.

Fig. 4. Inner connective tissue layer in the migraft part covered with parietal thrombosis on which epithelium is growing 23 days after operation. Masson staining. 400 ×.

Fig. 5. Inner and outher connective tissue layers joined by a connective tissue stripe running through the porous area of the prosthesis. 44 days after operation. Masson straining. 75 ×.

Fig. 6. A connective tissue stripe in the porous area together with a blood vessel. 44 days after operation. Masson staining. 190 ×.

Fig. 7. Inner layer formed from fibrous connective tissue closely associated with the prosthesis. 97 days after operation. Haematoxylin and eosin staining. 190 ×.

Fig. 8. Inner layer compact and composed of connective tissue with a very small number of cellular element, covered with regular epithelium. 201 days after operation. Haematoxylin and eosin staining. 190 ×.

Fig. 9. Inner layer a bit farther from the junction well-associated with the prosthesis, covered with pavement epithelium. 201 days after operation. Haematoxylin and eosin staining. 190 ×.

Fig. 10. A bundle of elastic fibres in the inner layer 272 days after operation. Haematoxylin and eosin staining. 400 ×.

STRESZCZENIE

Przeprowadzono badania histologiczne nad procesem wgajania się protez naczyniowych z teritalu. Doświadczenia wykonano na 10 psach, którym wszczepiano protezę w aortę brzuszną. Okres obserwacji wynosił: 12 godzin, 7, 14, 21, 23, 28, 44, 97, 201, 272 dni. Bezpośrednio po wszczepieniu w ścianie protezy jak również na jej powierzchni wewnętrznej i zewnętrznej odkładał się włóknik i elementy morfotyczne krwi. W dalszym etapie następowała organizacja warstw włóknikowych przez wrastanie elementów tkanki łącznej z końców aorty i z otoczenia protezy. Prawidłowo wgojona proteza pokryta była od wewnątrz

łącznotkankową warstwą, na którą narastał nabłonek. Od zewnątrz proteza również pokrywała się tkanką łączną. Te dwie warstwy były połączone pasmami tkanki łącznej przerastającej przestrzenie porowate protezy. Powstanie krwiaka wokół protezy wyraźnie hamowało prawidłowy proces wgajania się protezy.

SPIS RYCIN

Ryc. 1. Warstwa wewnętrzna utworzona z siatki włóknika silnie nasyconej elementami morfotycznymi krwi z dużym nagromadzeniem pałeczek, monocytów i limfocytów. 7 dni po operacji. Barwienie wg Massona. Pow. 400 ×.

Ryc. 2. Wrastanie elementów tkanki łącznej od zewnątrz pomiędzy poszczególne włókna teritalu. 21 dni po operacji. Barwienie hematoksyliną i eozyną. Pow. 400 ×.

Ryc. 3. Warstwa wewnętrzna w części środkowej protezy pokryta nabłonkiem prawie sześciennym z widocznym skrzepem przyściennym przylegającym do nabłonka od światła naczynia. 23 dni po operacji. Barwienie wg Massona. Pow. 190 ×.

Ryc. 4. Łącznotkankowa warstwa wewnętrzna w części środkowej protezy pokryta skrzepem przyściennym, na który narasta nabłonek. 23 dni po operacji. Barwienie wg Massona. Pow. 400 ×.

Ryc. 5. Łącznotkankowa warstwa wewnętrzna i zewnętrzna połączone pasmem tkanki łącznej przechodzącym przez przestrzeń porowatą protezy. 44 dni po operacji. Barwienie wg Massona. Pow. 75 ×.

Ryc. 6. Pasmo łącznotkankowe w przestrzeni porowatej wraz z naczyniem krwionośnym. 44 dni po operacji. Barwienie wg Massona. Pow. 190 ×.

Ryc. 7. Warstwa wewnętrzna utworzona z tkanki łącznej włóknistej ściśle związana z protezą. 97 dni po operacji. Barwienie hematoksyliną i eozyną. Pow. 190 ×.

Ryc. 8. Warstwa wewnętrzna blisko zespolenia składająca się z tkanki łącznej z bardzo małą ilością elementów komórkowych. Pokryta prawidłowym nabłonkiem. 201 dni po operacji. Barwienie hematoksyliną i eozyną. Pow. 190 ×.

Ryc. 9. Warstwa wewnętrzna nieco dalej od zespolenia dobrze związana z protezą, pokryta nabłonkiem brukowym. 201 dni po operacji. Barwienie hematoksyliną i eozyną. Pow. 190 ×.

Ryc. 10. Wiązka włókien sprężystych w warstwie wewnętrznej 272 dni po operacji. Barwienie hematoksyliną i eozyną. Pow. 400 ×.

РЕЗЮМЕ

Проведено гистологические исследования над процессом вживления сосудистых протезов из теритала. Опыты выполнено на 10 собаках, которым имплантировано протез в брюшную аорту. Период наблюдения равнялся 12 часов, 7, 14, 21, 23, 28, 44, 97, 201, 272 дня. Непосредственно после имплантации в стене протеза, как и на его внутренней и наружной поверхностях отлагался фибрин и морфо-

логические элементы крови. В следующий период имела место организация фибринного слоя путем врастания элементов соединительной ткани из концов аорты и окружающей среды протеза. Правильно вживленный протез был покрыт внутри слоем соединительной ткани, на которую нарастал эпителий. Снаружи протез тоже был покрыт соединительной тканью. Эти два слоя были соединены полосами соединительной ткани прорастающей свободные пространства протеза. Возникновение гематомы вокруг протеза четко тормозило закономерный процесс вживления протеза.

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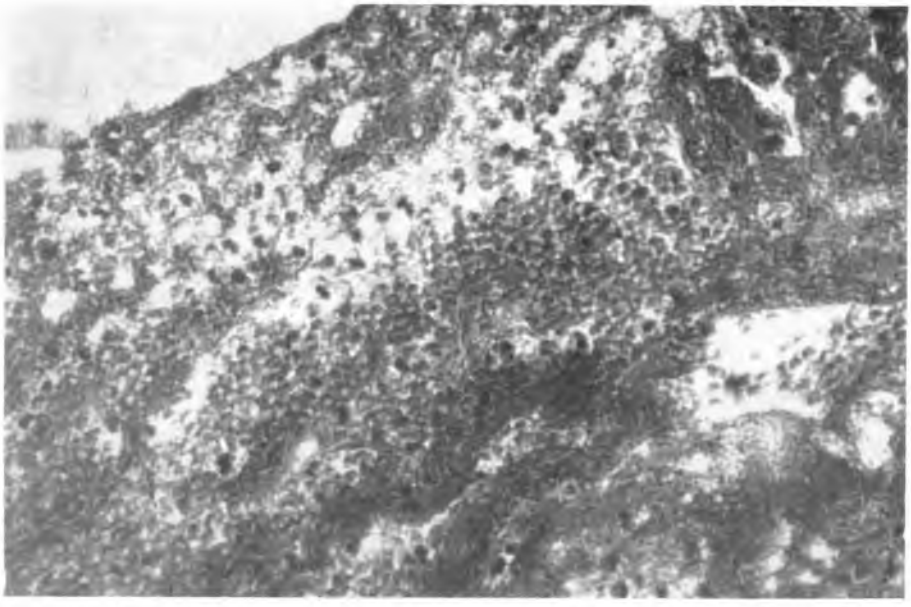

Fig. 1

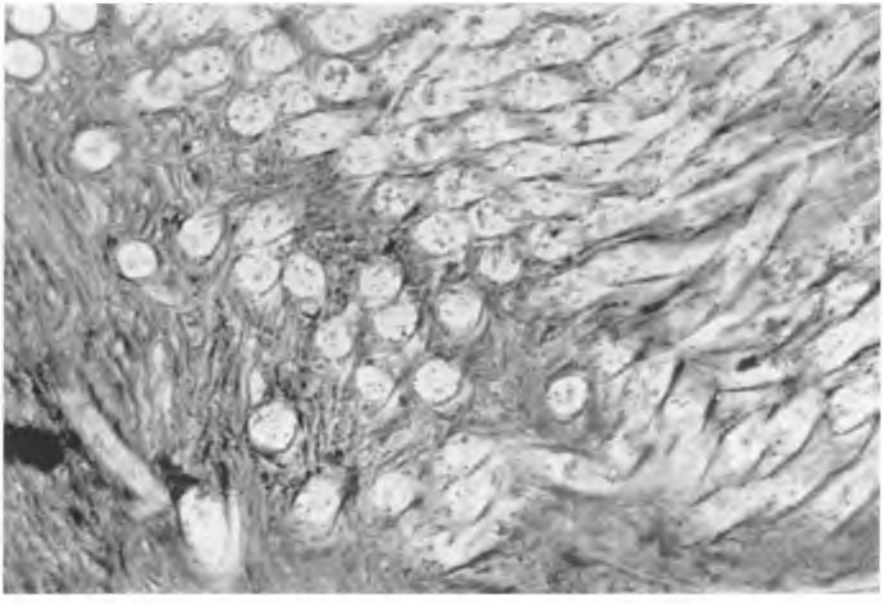

Fig. 2

Fig. 3

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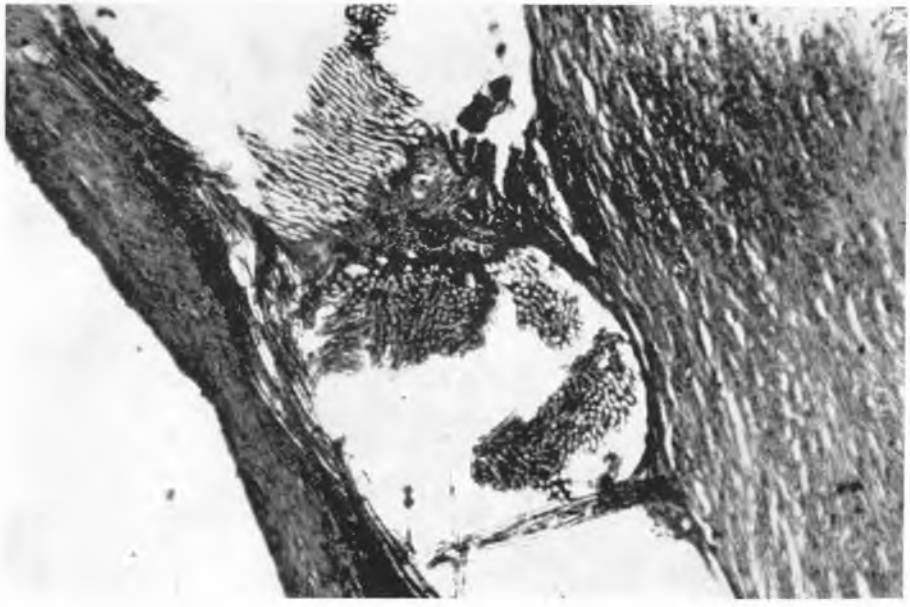

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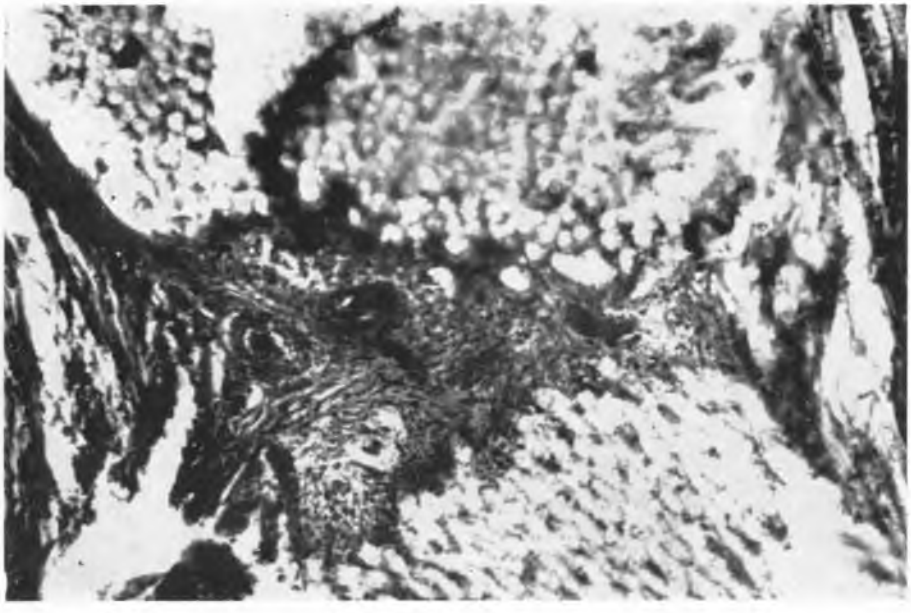

Fig. 6

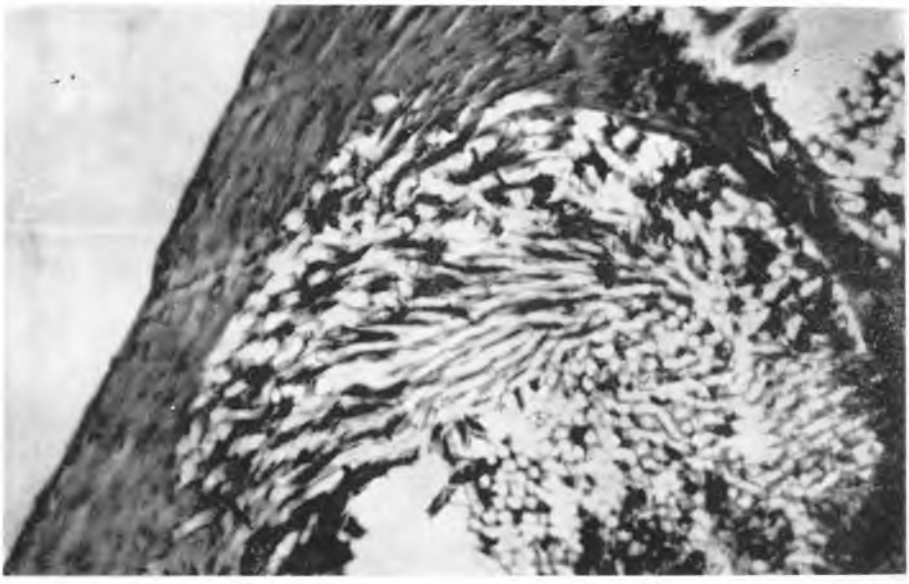

Fig. 7

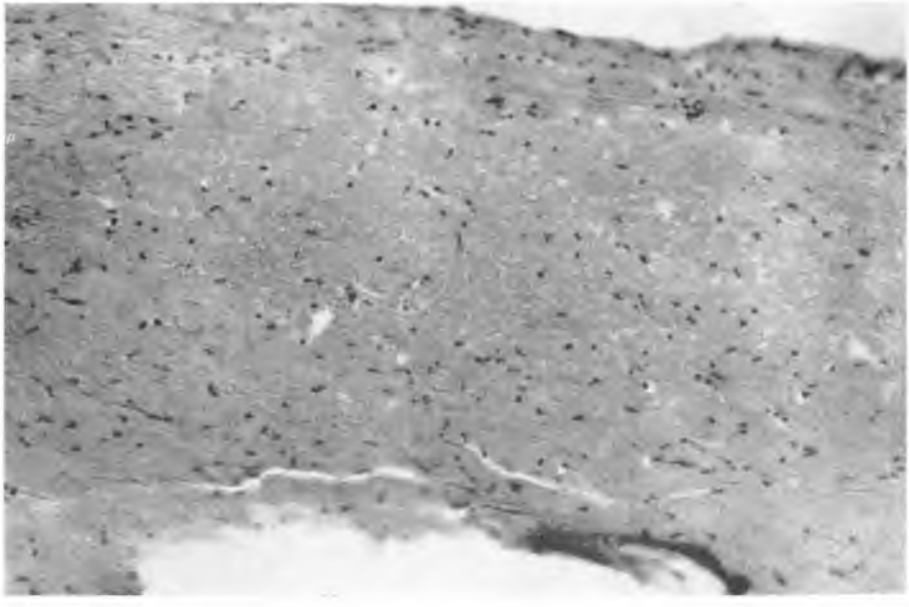

Fig. 8

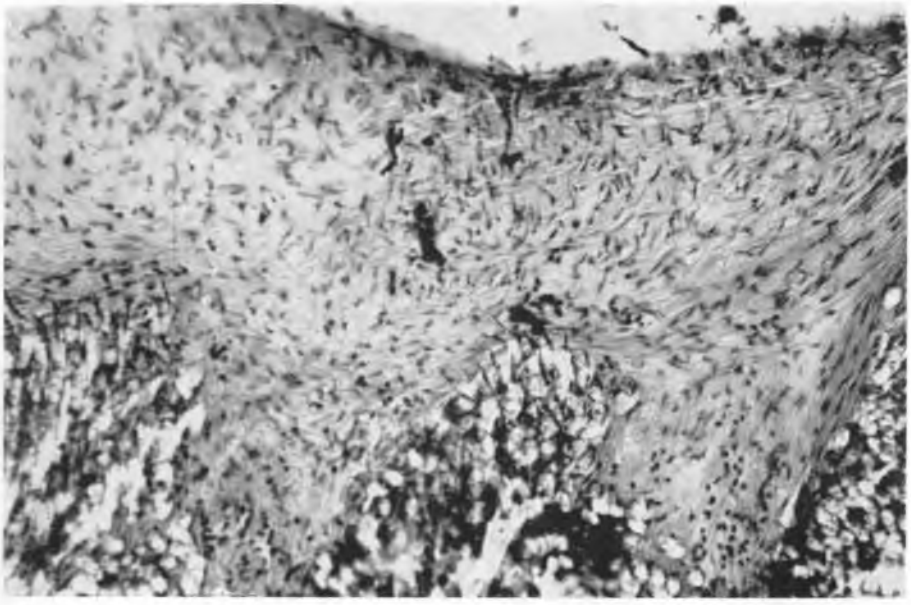

Fig. 9

Fig. 10