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TRAUMATIC PANNUS

II. Changes in Hydroxyproline after an Experimental Reconstruction of the Joint Surface

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The damage to joint surface (intraarticular fracture, damage to the intraarticular structure, transplantation) is followed by pannus formation. The primary avascular pannus, which fills all the defects of the cartilage in the first days after the operation, is later changed into the cellular and fibrovascular form. The last stage of this process is the plastic or lytic form of the pannus. The cytological changes accompanying the pannus formation after the reconstruction of the non-weight-bearing part of the joint surface by autogenous or homogenous graft have been described in the previous paper.

Similar cytological changes have been observed in the reparation of artifical defects in autografts (Fiala & Bartoš 1967). We found that the drilled defects were filled with erythrocytes dispersed in the fibrin net soon after transplantation. The granulation tissue formed at the bottom grew up to the surface of the defect and gradually replaced the original content of erythrocytes and fibrine. Later this granulation tissue was differentiated into the cancellous bone with fat or blood marrow spaces and into the fibrocartilage at the surface.

In this paper the attention was focused to one of the most important components taking part in the process of tissue healing and pannus formation—collagen. This protein can be readily identified by the amino acid hydroxyproline which is found almost exclusively in collagen.

METHODS

Ten adult mongrel dogs were in our experiments. The anterior non-weight-bearing portion of the distal articular end of the femur in the form of a osteocartilaginous autograft was transplanted. Four holes 3 mm in diameter were drilled in each graft

before transplantation. The transplants were fixed to the bed with one loop of stainless steel wire. The synovial fluid, contents of the artificial holes (plugs) and pannus formed on the sides of the graft and at the junction of the graft cartilage with the periosteum of the bed, were removed 3, 17, 14 and 28 days after operation.

Samples were dried in an oven at 80° C to the constant weight. The dried tissue was hydrolyzed for 16 hours in 6N HCl at 105° C in tubes sealed under nitrogen. Hydroxyproline was determined by the method of Stegemann (1958). The values were converted to collagen equivalents using the Neumann & Logan (1950) factor of 7.46. Each point in Figure 2 presents the average of four pools from four animals. All differences (pannus:plugs) are highly significant ($p>0.01$). Collagen forming has been morphologically observed in the unstained sections (10 μm) by the polarization microscope at crossed polaroids.

RESULTS

Figure 1 shows the changes in the dry weight of tissues and synovial fluid during the experiments. The dry weight of plugs was higher 3 days after the operation; other changes were not significant.

Figure 2 demonstrates the changes in collagen content. The synovial fluid contained only traces of hydroxyproline and was therefore omit-

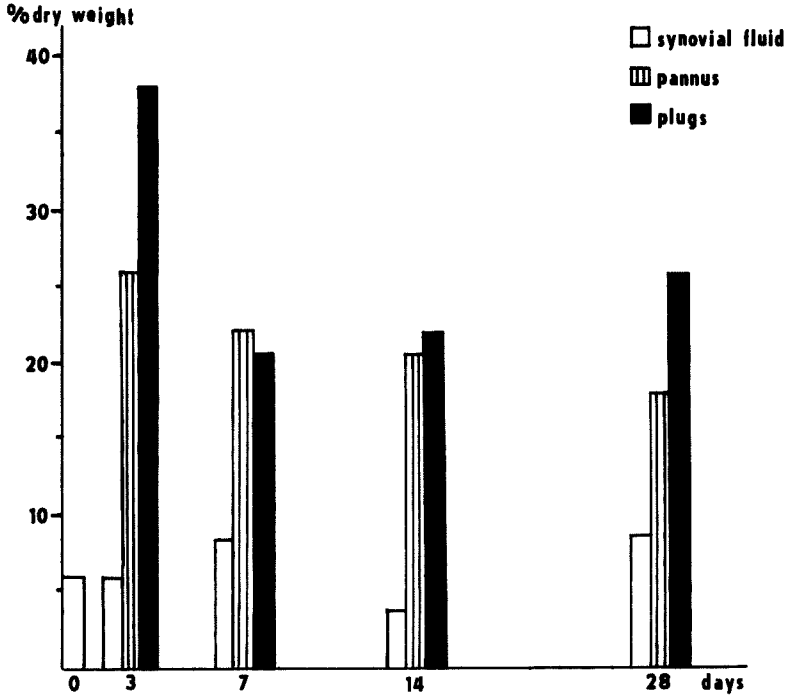

Figure 1

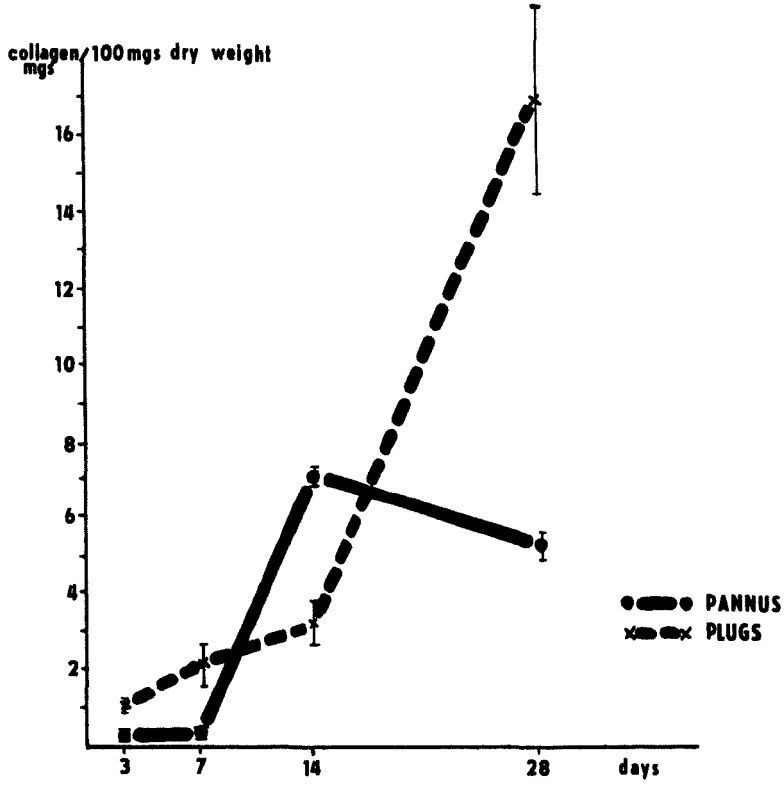

Figure 2

ted in the picture. The amount of collagen in pannus was very low for the first 7 days after the operation; then it markedly increased and was maintained on the same level during the second half of the experiment. In the plugs the hydroxyproline content was increasing throughout the experiment, first slowly, later very rapidly.

DISCUSSION

The results of chemical assays of collagen during the formation of traumatic pannus and healing of artificial defect in osteocartilaginous graft agree with cytological and morphological findings.

In the first week after transplantation in the plugs and in the pannus itself there is very little amount of collagen because the vascular pannus is mostly composed of erythrocytes and fibrin. Although there are

no collagen-forming cells—fibroblasts, respectively fibrocytes—we have found a certain amount of collagen in these tissues. This percentage of collagen is significantly higher ($p>0.01$) in plugs, which can be explained by the presence of tiny fragments of bones and cartilage. The surface of the plugs closing the holes against the joint cavity is highly birefringent. It is difficult to decide if there are collagenous fibres or remnants of bone or cartilage formed during the drilling of the holes in the graft.

In the second week after the transplantation the increase of collagen amount in the pannus shows that the avascular form of the pannus has changed into cellular and fibrovascular tissue. A rather stabile amount of collagen in the pannus 28 days after transplantation can be explained by the incomplete pannus separation after opening the joints. In this time the pannus adheres strongly to bone or cartilage and it is not possible to remove the whole pannus without damaging the bottom. For this reason only the superficial and younger parts of the pannus containing less collagen were taken and then examined.

The slow increase in collagen occurring in plugs for 14 days after the transplantation and the later rapid increase fully correspond with the morphological changes during the reparation of defect in the osteocartilaginous graft (Fiala & Bartoš 1967). Fourteen days after the transplantation the tissues filling the defect may be divided into three zones: The deepest layer is formed by the granulation tissue with the osseous framework at the bottom. The middle layer contains a lot of macrophages and erythrocytes with fine fibrinous fibres. The superficial layer is composed from the net of fibrinous fibres with erythrocytes among them. On the surface of this zone there is a dense net with doubly refractive contents. The microscopical arrangement of the plug shows that the collagenous fibres can be found only in the basal zone. On the contrary, the pannus situated on the side of the graft is penetrated by the granulation tissue in its whole thickness during this time.

Four weeks after the transplantation the defect in the graft is filled up either with granulation tissue or with bone trabecullae, for which reason the amount of collagen in plugs is so high.

The determination of hydroxyproline in the formation and ageing of the pannus supported the macroscopic and microscopic findings after the transplantation of osteocartilaginous graft. This determination verified as well the division of the pannus into the basic two forms: the primary avascular form which is mainly formed by fibrin

and erythrocytes, and the cellulovascular form that is presented on the other hand by the collagen-forming granulation tissue.

SUMMARY

The authors transplanted the anterior portion of the distal articular end of the femur in the form of perforated osteocartilaginous autograft in dogs. The amount of hydroxyproline in the pannus forming either on the sides of the graft or in the artificial defects was determined. The authors found that in the first week the content of hydroxyproline in the pannus was minimal and increased in the second week after the transplantation when the primarly avascular pannus was transformed into the cellulo-fibrovascular form.

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