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## EARLY CHANGES IN THE COMPOSITION OF RABBIT ARTICULAR CARTILAGE FOLLOWING EXPERIMENTALLY PRODUCED INTRA-ARTICULAR FRACTURES

*A Histological and Thermoanalytical Study*

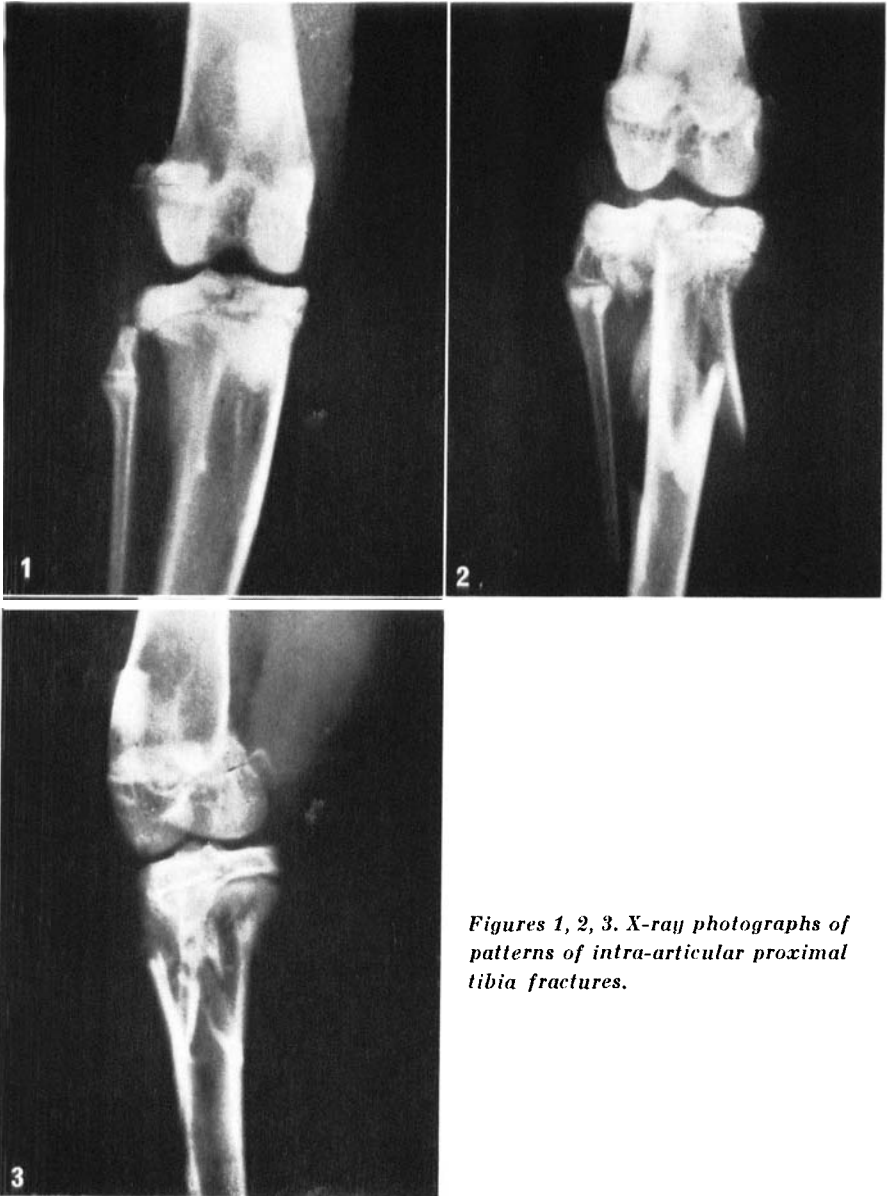
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The treatment of intra-articular fractures remains an unsolved problem, until the reasons for the development of posttraumatic arthritis are cleared up satisfactorily. Based on numerous clinical observations of intra-articular fractures, factors such as surface incongruity, joint instability, deformation of the axis of weight transmission, prolonged immobilisation, and advanced age of the patient are already well appreciated, but other apparent factors accounting for the high incidence of post-traumatic arthritis are not yet fully understood. Some authors, on the basis of the effects of haemarthrosis, lipoarthrosis, enzymatic breakdown and malnutrition in basic experiments, emphasize the importance of initial joint trauma (Schulitz et al. 1973). The object of the work described in the present paper was to study the possible early damages taking place in the structure and composition of articular cartilage as a result of joint trauma. To this end intra-articular fractures were produced experimentally and the cartilage changes investigated by histological, histochemical and thermoanalytical methods.

### MATERIALS AND METHODS

Thirty-five 6-month-old rabbits were used in the experiments. The use of not fully mature animals avoided possible degenerative changes related to aging, and the relative thickness of the articular cartilage provided more material for processing. The animals were housed under normal conditions and kept on stock diet. Under



*Figures 1, 2, 3. X-ray photographs of patterns of intra-articular proximal tibia fractures.*

intravenous barbital anaesthesia intra-articular fractures were produced in the right tibial condyles of each animal. By means of a modified gripping tool, bilateral compression was exerted on the condyles. Squeezing the spongy bone of the condyles, the padded arches of the tool were closed to a standard width of approximately 6 mm. The resulting intra-articular fractures were verified in all instances on X-ray

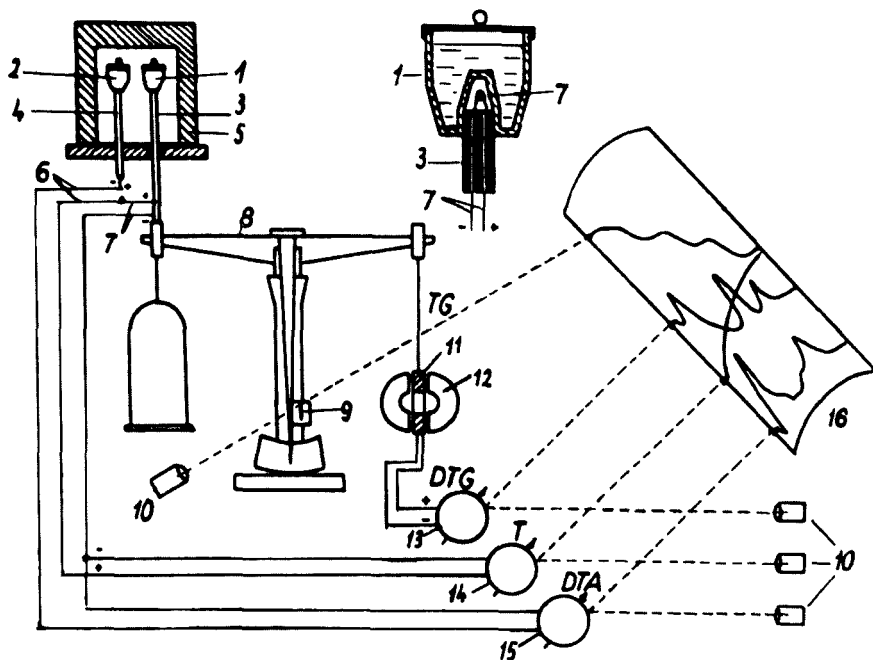


Figure 4. Structure of the derivatograph. 1—crucible for the sample, 2—crucible for the inert substance, 3, 4—porcelain tube, 5—electric furnace, 6, 7—thermocouples, 8—balance, 9—optical slit, 10—lamps, 11—coil, 12—permanent magnets, 13, 14, 15—galvanometers, 16—photographic paper.

photographs taken of the injured knee joint (Figures 1, 2, 3). The injured limbs were left free to move. The opposite knee joints served as controls.

At appropriate time intervals (3 days, 1, 2, 3, 4, 7 and 8 weeks after the induction of fracture) 5 animals were sacrificed, respectively. Immediately after death, the proximal part of the tibia was isolated and a 2 mm thick osteochondral layer was removed from the largest extension of the injured condyles for histology. Decalcified paraffin sections were prepared and stained with HE and PAS Alcian blue. The articular cartilages of the femoral and tibial condyles were excised and subjected to thermal analysis.

In order to follow the healing process of the fractures, callus samples from each stage of every experiment were collected from the fractured proximal tibia and processed by the thermoanalytical method.

Thermal analysis was carried out using a Paulik-Paulik-Erdey (Paulik et al. 1958, Paulik & Paulik 1971) MOM Derivatograph (Figure 4). The instrument measured and recorded simultaneously the weight change (TG curve), rate of weight change (DTG curve) and enthalpy change (DTA curve) as functions of temperature in the same sample.

The material, approximately 50 mg, was weighed into a platinum crucible. The heating rate was 2°/min up to 900° C. The quantitative determination was carried

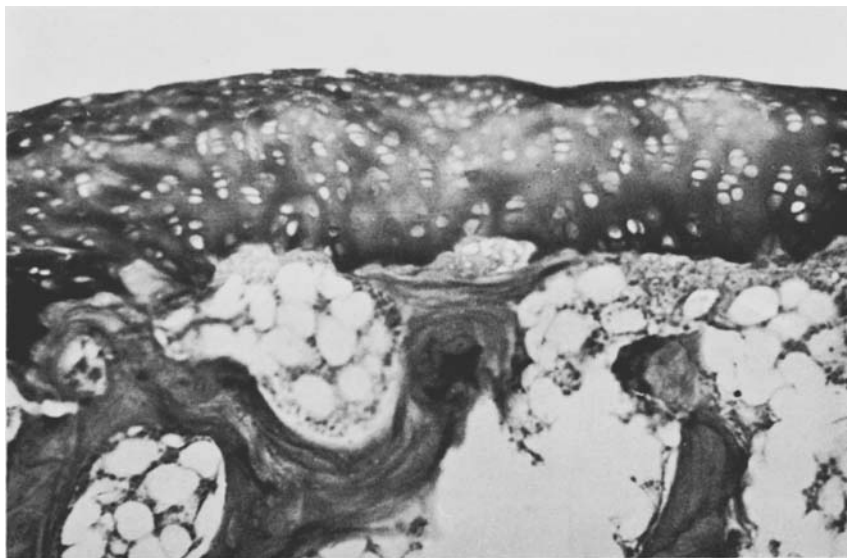
out on the basis of the TG curve, but with the help of the DTG curve, which shows transformations accompanied by weight change and their characteristic temperatures. DTA curves served for the qualitative evaluation of processes which were connected with weight and heat effects of a parallel nature but of opposite sign. In the derivatograph the evolution of the structurally bound water content, the decomposition of glycosaminoglycans (Bihari-Varga 1971 a) and of proteins (Bihari-Varga 1971 b) took place at well-defined characteristic temperatures and thus could be studied individually. Inorganic compounds turned up in the form of a thermostable residue.

## RESULTS

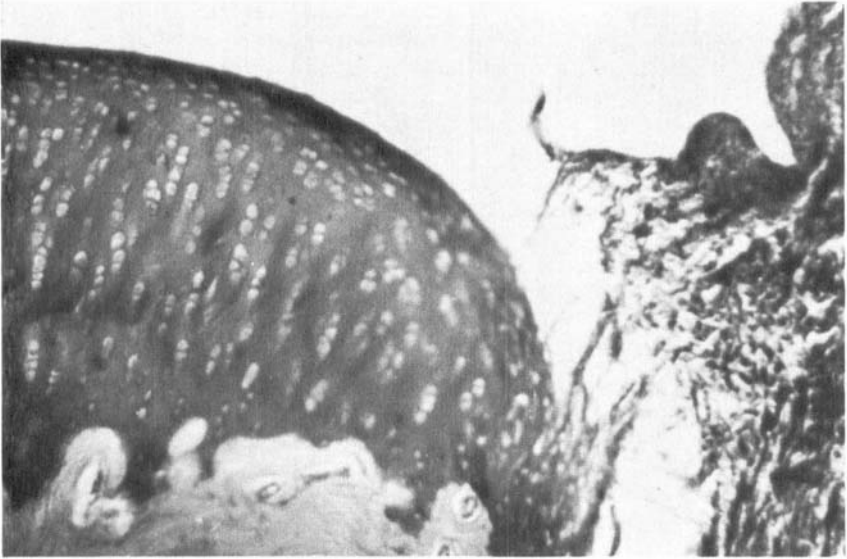
### *Morphological findings*

Gross examination of the injured joints on the 3rd day after fracture revealed massive haemarthrosis which presented after 1 week as small blood clots and which from the 2nd week after injury was absent. Fragmentation and displacement of the tibial articular surface was noted in seven cases out of the 35. From the 2nd week granulation tissue could be seen, but even in the group sacrificed 8 weeks after fracture original cartilage cover was clearly separable. On the femoral condyles the cartilage surfaces showed basically no variations.

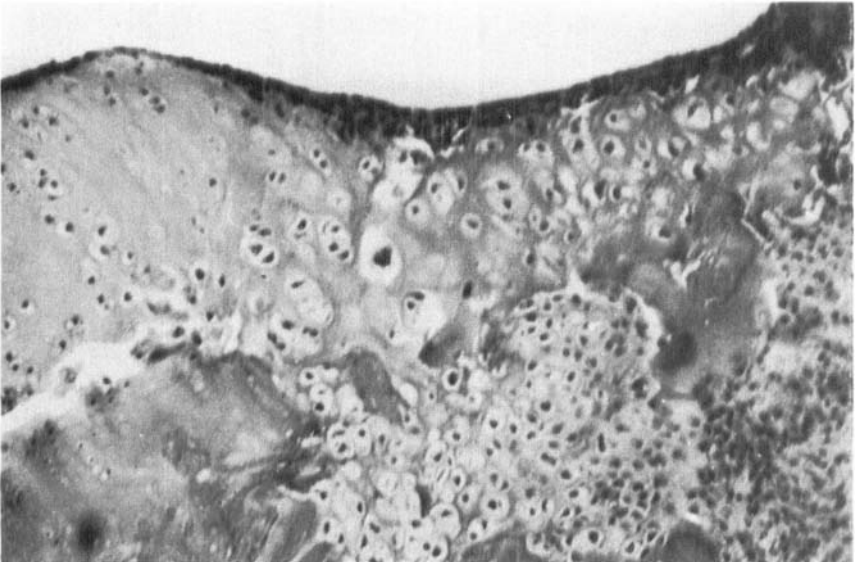
On microscopic examination of sections from the 3rd day (Figure 5)



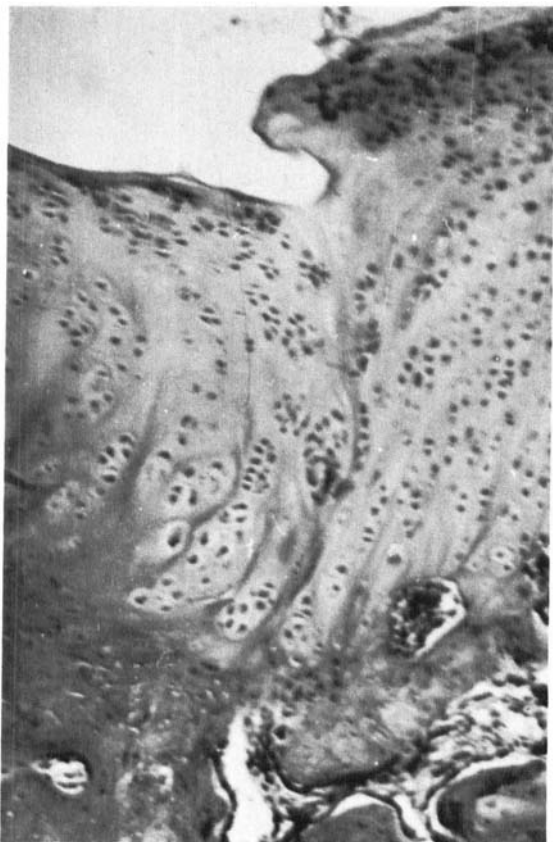
*Figure 5. Articular cartilage from the knee joint of a rabbit, 3 days after injury. Slight loss of matrix staining. (Periodic acid–Shiff and Alcian blue,  $\times 12$ ).*



*Figure 6. Articular cartilage from the knee joint of a rabbit. 1 week after injury. Deficient metachromasia. (Periodic acid-Schiff and Alcian blue,  $\times 20$ ).*



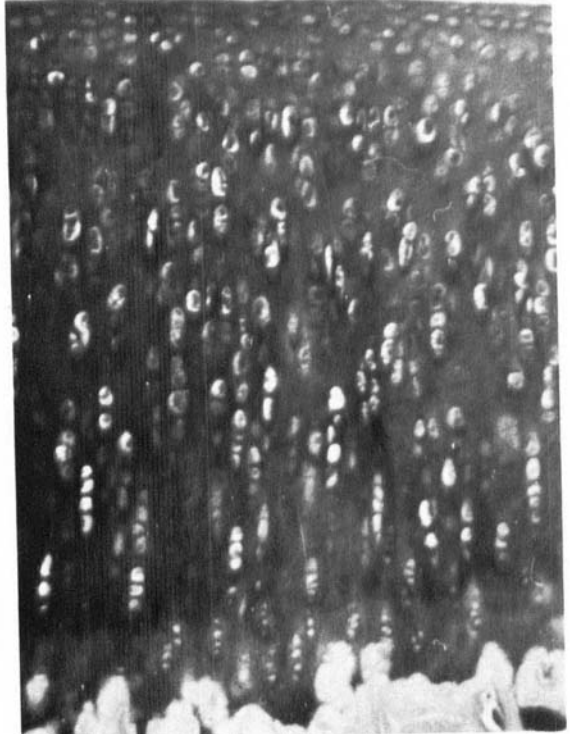
*Figure 7. Articular cartilage from the knee joint of a rabbit. 4 weeks after injury. Site of osteochondral fracture. Depressed articular surface covered by pannus. Fibrocartilaginous regeneration. Chondrocyte clusters. (Haematoxylin and eosin,  $\times 20$ ).*



*Figure 8. Articular cartilage from the knee joint of a rabbit, 4 weeks after injury. Stepwise depression of the cartilage surface. Chondrocyte clusters. (Haematoxylin and eosin,  $\times 40$ ).*

and 1st week (Figure 6) a slight decrease in matrix staining appears to occur in the PAS Alcian blue stained sections, but no essential difference from the normal is noted. Starting from the 2nd week signs of regeneration, mitotic figures with chondrocyte clusters, appear in the original cartilage cover. The slide from the group sacrificed 4 weeks after the fracture shows a site of injury on the articular surface (Figure 7). Under the original, impressed cartilage cover a subchondral horizontal cleft is visible. The site of bony fracture is filled up with cartilaginous callus. The mechanically deranged and compressed original cartilage layer is covered by a sheet of pannus. Adjacent to the injured part, chondrocyte clusters appear in the original cartilage. A similar appearance of the original cartilage is seen on a section from the same group, with stepwise depression of the original cartilage surface (Figure 8). A cartilage sample from the group sacrificed 7

*Figure 9. Articular cartilage from the knee joint of a rabbit, 7 weeks after injury. Almost normal cartilage structure and metachromasia. (Periodic acid-Schiff and Alcian blue,  $\times 40$ ).*



weeks after injury, with normal acid mucopolysaccharide pattern on the PAS Alcian blue stained section shows an almost normal appearance (Figure 9). On a section 8 weeks after injury, the original articular cartilage in a depressed and buried position is still well recognizable, with normal tide mark and with chondrocyte clusters (Figure 10).

#### *Results of thermal analysis*

Thermal analysis showed slight, not significant alterations in the chemical composition and structure of the cartilage tissue of the injured joint within the first 8 weeks of the healing period (Figure 11).

*Figure 10. Articular cartilage from the knee joint of a rabbit, 8 weeks after injury. The articular cartilage is overgrown by fibrous tissue. The original cartilage structure is well recognisable. (Haematoxylin and eosin,  $\times 20$ ).*



#### *Demonstration of fracture healing by thermal analysis*

Thermoanalytical studies on callus (Figure 12) indicated an increase of glycosaminoglycan content compared to the unfractured bone, in good agreement with data from the literature. Glycosaminoglycan concentration decreased gradually during calcification. Structurally bound water content showed a maximum as a function of time, reaching its peak value at about 3 weeks after injury. The composition of the regenerating bone resembled that of the unfractured bone in the 8th post-traumatic week, and thus the healing process could be regarded as completed.

#### DISCUSSION

As described previously (Bihari-Varga et al. 1975, Farkas et al. 1974) in accordance with the results of numerous investigators, early cartilage lesions were found to be strikingly similar, irrespective of the

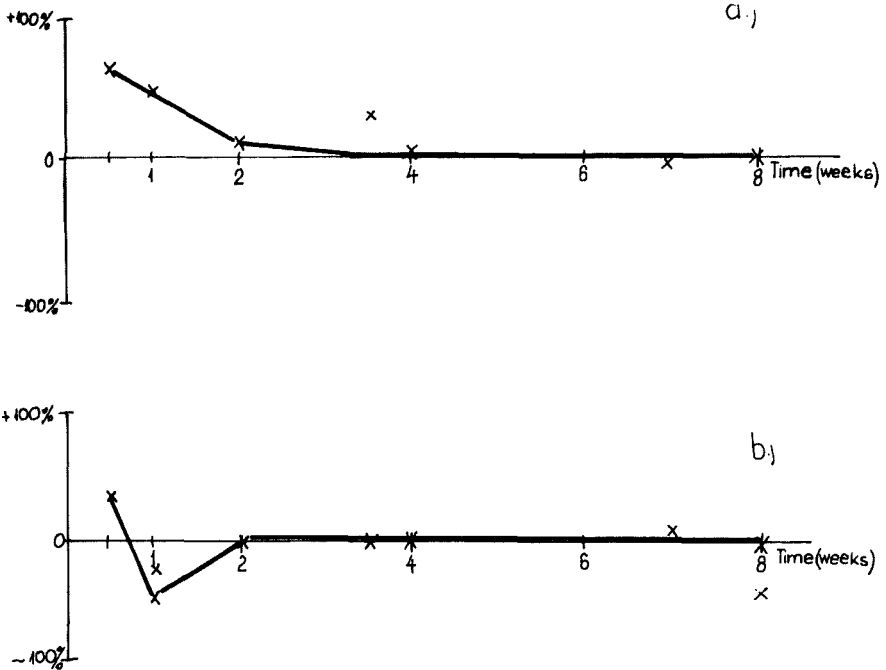


Figure 11. Percentage changes in structural water content (a) and glycosaminoglycan content (b) of rabbit articular cartilage as a function of time after experimental induction of fracture. Data are referred to those measured in the corresponding cartilages of the control extremity.

method used for the production of degenerative alterations, viz. a gradual deterioration in the course of the first week, resulting in a significant decrease in the proteoglycan content of the matrix without change in the collagen, followed by a complete repair of the composition of the cartilage towards the 6th week.

In the present experiments a similar pattern of aspecific early reaction of the cartilage of injured joints is suggested by the histological sections. The morphological appearance, however, never recovers to normal during the early phase. The alterations are focal in nature, as revealed by histology, and so slight that no significant alterations in the average composition of the cartilage could be measured by thermal analysis.

For a possible relationship with late arthrosis, the experimental results of Hjertquist & Lemperg (1971) should be examined. After having produced osteochondral defects in the articular surface of rabbit femoral heads, these authors could observe no degenerative

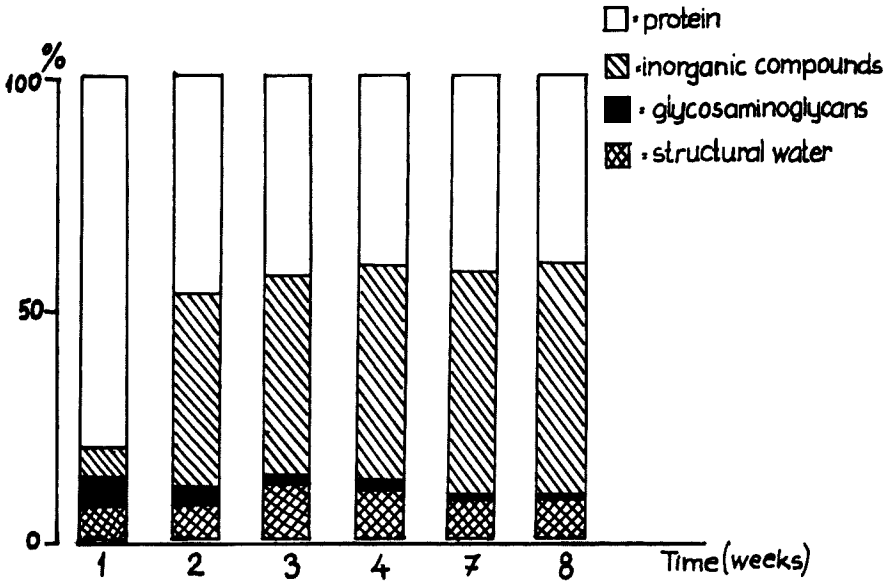


Figure 12. Changes in the composition of callus.

changes in the articular cartilage outside the defect area over periods up to 20 weeks. At 40 and 52 weeks, however, some loss of metachromatic material and superficial fibrillation occurred, with a tendency towards lower values of total hexosamines.

Clinical observations indicate a similar slow development of post-traumatic arthritis with rising incidence even more than 2 years after trauma.

From our experiments it can be concluded that there is no gradual deterioration in the structure and composition of articular cartilage after intra-articular fracture, at observation times up to 8 weeks, which would lead directly to irreversible changes consistent with arthritis. Within this period the early effects of trauma subsided.

S U M M A R Y

Intra-articular fractures were produced in rabbit knee joints. The articular cartilage of the injured joints was processed histologically and analysed by a complex thermoanalytical method. It was demonstrated that intra-articular fractures in the early stages have essentially no irreversible damaging effects on the composition of articular cartilage of injured joints.

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