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EFFECTS OF EXERCISE ON ADULT ARTICULAR CARTILAGE

AN EXPERIMENTAL STUDY ON GUINEA-PIGS WITH RELEVANCE TO THE CONTINUOUS REGENERATION OF ADULT CARTILAGE

BY

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Preface

During my work as an assistant at the Institute of Anatomy of Uppsala University, my chief, Professor D. E. Holmdahl, awakened my interest in questions concerning the morphophysiology of the joints.

After some preliminary investigations I found that the problem which is dealt with in this paper had not been treated before; so the present investigation was started.

I am profoundly indebted to Professor Holmdahl, at whose institute this work was carried out, for his never failing interest and all his invaluable help.

My sincere gratitude to Professor G. Dahlberg for sage advice concerning statistical problems and for profitable discussions.

Professor B. E. Ingelmark has given me valuable support through sound criticism and encouragement, for which I am very grateful.

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Finally, I wish to thank my wife for helping me with this work throughout the years and for her continuous encouragement.

Uppsala, November, 1950.

Josef Sääf.

Review of Literature

The literature of cartilaginous tissue is at present very comprehensive. This review, however, is confined mainly to the works dealing with articular cartilage and of importance to the present investigation.

Owing to the fundamental works of several scientists, such as Tillmanns (1874, 1877), Hammar (1892), Hultkrantz (1897), Hansen (1905), Lubosch (1910, 1927, 1938), Benninghoff (1922, 1924, 1925), and Schaffer (1930), we have obtained good knowledge of the structure of hyaline cartilage, and accordingly also of articular cartilage.

In his works of 1925 Benninghoff compiled his own results and those of earlier research workers concerning the structure of the articular cartilage. He was also able to bring together the individual elements of the cartilaginous tissue into functional systems. Thus Benninghoff has in a decisive manner contributed to increasing our knowledge of the connection between the structure of the articular cartilage and the function of the joint.

For this reason, and since Benninghoff's terminology will be used in the present investigation, it appears to be justified to give a summarized account of his view concerning the structure of the articular cartilage and its function.

The articular cartilage is divided into the calcified and the uncalcified cartilage. The latter, in its turn, consists of three zones. The superficial *tangential zone* next to the joint-cavity is thin, having flattened cells parallel with the articular surface. Under this we find the *transitional zone*, having more rounded cells often in clusters, and finally the *radial zone*, which is contiguous to the calcified cartilage.[1] In this zone the cells are often protracted—each having

[1] Sometimes Benninghoff speaks of the calcified cartilage as a part of the radial zone, but alternatively he also uses the above designations (Ben-

its long axis at right angles to the articular surface—, or arranged in vertical rows.

The cartilage is made up of a cellular and an intercellular component, the latter of which consists of collagenous fibrils embedded in hyaline substance. BENNINGHOFF has succeeded in elucidating the course of these fibrils. From the base of the calcified layer the fibrils pass through the radial zone at right angles to the articular surface. They deflect in the transitional zone, run a short distance, in the tangential zone, parallel with the articular surface, and then down through the cartilage, finally to become fixed again in the calcified layer.

These arcades of collagenous fibrils constitute one of the two functional systems of the structures of the articular cartilage. The other consists of the so-called chondrons, by which BENNINGHOFF means bodies having elasticity of compression and consisting of one or more cartilage-cells enveloped by a layer of concentrically-running collagenous fibrils.[1] The chondrons are located between the bundles of the arcades, and their most superficial fibrils seem to run into the arcade-system (BENNINGHOFF, 1939).

It may be mentioned that LUBOSCH (1927, 1938), BORMUTH (1933), and BUCHER (1942), amongst others, partly have a different view concerning the course of the fibrils in the hyaline cartilage. Their investigations, however, are generally carried out on cartilage covered with perichondrium, so their results do not seem to be immediately applicable to the articular cartilage.

The collagenous fibrils are bound together by the hyaline substance, which also masks the fibrils and gives a homogeneous appearance to the intercellular component. This substance also contributes to making the articular surface relatively smooth.

NINGHOFF, 1925, 1939). For terminological reasons I have chosen to employ the latter in the present investigation.

The German designations of the various zones and their corresponding translations into English are presented here: —

Die Tangentialschicht, or *Tangentialfaserschicht* = the tangential zone.

Die Übergangszone = the transitional zone.

Die Radiärzone, or *Radiärfaserschicht* = the radial zone.

[1] The designation "chondron" used by BENNINGHOFF has been employed without being translated.

On account of its structure and composition, the articular cartilage is compressible and elastic. When the articular surfaces are pressed against each other, the cartilage is compressed and transformed, the contact-surface between the articular cartilages becoming larger, and the load per unit of surface less. BENNINGHOFF has also pointed out that the compression of the articular cartilage is accompanied by a tangential displacement of the tissue from the centre of the compressed area towards the periphery. This displacement involves mainly the two superficial zones of the uncalcified cartilage, thus affecting the shape of the fibril-arcades. This change of shape, in its turn, causes a more transverse compression of the chondrons between the bundles of fibrils, whereby the pressure on the articular surface is consequently transferred to these elastic bodies.

The superficial tangential zone possesses an appropriate tensile strength owing to the collagenous fibrils running parallel with the articular surface within this zone.

Consequently, the articular cartilage is an extraordinarily specialized tissue and so constructed in detail that it appears to be adequate to functional demands.

On the other hand, the normal functioning of a joint, with its movements and intermittent compressions of the cartilage, is of vital importance to the continued existence of the latter. Both clinical experience and experimental investigations have given evidence of this. Thus degenerative changes have been found in the articular cartilage after some time of immobilization of a joint (REYHER, 1873; MOLL, 1886; NATHAN and STRANG, 1909; MÜLLER, 1924, 1929; DEBRUNNER, 1925; ELY and MENSOR, 1933). Moreover, a continuous pressure on the articular cartilage, e.g. by tendons, cartilage, or bones, can lead to its destruction (WEHNER, 1923; MÜLLER, 1924, 1929; KOCH, 1927; SCAGLIETTI, 1929—30).

MÜLLER (1929) considers that the cause of these degenerative changes is to be sought for in an impairment of the nutrition of the articular cartilage. In fact, he is of the opinion that the nutrition of the articular cartilage is directly dependent on the functioning of the joint. He assumes that the inflow of fluid, necessary for nutrition, into the articular cartilage is especially promoted by the intermittent compressions in the normal functioning of the joint.

The correctness of this view has been corroborated through experimental investigations, by INGELMARK and SÄÄF (1948), on joints from freshly killed rabbits; and by EKHOLM (personal communication) using radioactive gold in a study on living rabbits. By x-ray studies, INGELMARK and EKHOLM (1948 and personal communication) have proved that the thickness of the articular cartilage increases rapidly with an intensification of the function of the joint, which they regard as depending on an increased inflow and content of fluid in the cartilage.

The intimate connection between the function of the joints and their morphological differentiation has been the object of investigations and discussions in literature.

During the latter part of the previous century rather much interest was devoted to the question as to whether the shape of the articular ends of a joint is determined by heredity or by mechanical conditions in embryonic life. It has proved to be difficult to draw a definite limit between the influence of heredity and that of function on the formation of the joints. Research workers, however, seem to have agreed that the shape of the joints is largely determined by hereditary factors, whereas their detailed differentiation depends on the formative influence of external factors. For more detailed accounts of this problem, we refer to FICK (1904), LUBOSCH (1910), and JUNGHANS (1939).

The formation of the tissue of the articular cartilage seems to follow the same principle. WEICHSELBAUM (1877) pointed out that children have a more indifferent articular cartilage, where there is, as yet, no possibility of distinguishing the architecture typical of adult articular cartilage.

Subsequent experimental investigations have also clearly shown that the function has an affect on the differentiation of the articular cartilage in growing animals.

RETTERER (1908) amputated one of the thoracic limbs in 1—2 month-old guinea-pigs. The distal part of the brachium and the antebrachium were removed. Consequently, the other limb came to be loaded more than what is normally the case, whereas the stump of the operated limb was unloaded, but movable. The operated animals lived for 4, 6, 8 months, and 1, 2, and 3 years. The number

of animals is not indicated. The results recorded seem to be based on the measurements carried out on some individual animals. The hyperactive and the inactive shoulder-joint were respectively the objects of a histological examination; and a comparison was made between each of these and the articular cartilage from a shoulder-joint of an adult guinea-pig which had lived normally while growing up. A hyperactive joint revealed a thicker articular cartilage, hypertrophy, and hyperplasia of the cells as well as broader streaks of intercellular substance. The inactive joint, on the contrary, had a thinner articular cartilage, smaller cells, fewer rows of cells, and less intercellular substance.

The same problem has been treated by SÄÄF (1941) in a study on the affect of the function on the articular cartilage of growing guinea-pigs. Some animals were exercised daily in a special apparatus from the age of three weeks up to approximately that of five months. Between these periods of exercise they lived in spacious cages. Other animals, which were not exercised, were confined to very small cages during the same length of time. The histological sections of their hip-joints were subjected to a microscopic examination. Within the weight-bearing part of the femur-head of exercised animals the cartilage revealed larger cells, a greater number of cells per chondron, and more intense basophilic intercellular substance than within the corresponding area of unexercised animals. The whole circumference of the articular cartilage of the latter had a more uniform and indifferent appearance. These results are solely based on direct comparisons between the histological sections, the material being not sufficiently large for a statistical treatment.

These two studies support the assumption that the function influences the differentiation of the articular cartilage during the period of growth. In a comprehensive experimental investigation on growing rabbits, however, HOLMDAHL and INGELMARK (1947, 1948) have presented data of a more convincing character.

The material comprised 61 rabbits divided into three groups. One group was subjected to systematic exercise from the age of six weeks, whereas the remaining two were not exercised. One of these groups lived in spacious cages, and the other in small ones. The animals were killed at the age of 24 weeks, and their shoulder-, el-

bow-, wrist-, hip-, knee-, and ankle-joints were the object of a quantitative micromorphologic analysis. The findings registered were subjected to statistical treatment, comparisons being drawn between large and small joints, between condyles and sockets, as well as between the various groups.

As the results of the comparison between the various groups are of such a nature as to elucidate the influence of the function on the formation of the articular cartilage, our account will here be limited to these results.

Firstly, as to the thickness of the uncalcified cartilage:—It was in large joints 23—45 per cent thicker in exercised animals than in unexercised. But, again, the two unexercised groups revealed differences themselves in this respect. The animals that had lived in spacious cages generally had a thicker uncalcified cartilage than those having lived in small. These differences were, however, not statistically significant. On the contrary, the varying function of the joints did not seem to have had any influence on the thickness of the calcified cartilage.

The analysis of the uncalcified cartilage also revealed that exercise had brought about an absolute increase of both the cellular and the intercellular substance. This increase, however, was approximately four times as great in the intercellular substance as in the cellular, which resulted in the fact that the cellular substance, in spite of its absolute increase, was nevertheless relatively decreased in the exercised animals.

The cellular component was also studied with regard to the sizes of the cells and the number of cells per chondron. A comparison between the various groups of animals as to the number of cells per chondron revealed in practice only probable differences. So the authors summarized these results as follows: "There is reason to assume that an increase in the functional strain of the cartilage stimulates an increase in the number of cells per chondron."

A study of the histological sections, and comparisons between photographs of these, gave a definite impression that the articular cartilages of exercised animals had larger cells than those of unexercised. But, "probably on account of the methodology applied, the present examination has not given statistically significant dif-

ferences concerning the size of the cartilage cells in the various groups."

The investigations above indicated that an intensification of the functioning of a joint no doubt influences that increase of cartilaginous substance which is connected with the growth of an individual. Consequently the function contributes to the definitive formation of the articular cartilage.

On the other hand, the question as to how the tissue of the articular cartilage of an adult reacts on an increased function, has not been the object of systematic investigations. Earlier works, however, have treated problems intimately relevant to this question. I primarily refer to the relationship between the function of a joint and the wear and tear of the articular cartilage, on the one hand, and the capacity of the cartilage to continuous regeneration, on the other.

That there is normally a wear and tear of the articular cartilage as an effect of the function of the joint, has early been advanced by several workers (BRINTON, 1847—49; TILLMANNS, 1874; OGSTON, 1876; HAMMAR, 1892). This view has later been corroborated, by LUBOSCH (1910), MÜLLER (1929, 1930), and HULTÉN and GELLERSTEDT (1940) amongst others. As a reason for the existence of such a process it has been pointed out that the surface of a normal articular cartilage is more or less rough, showing very small excavations and "splinters" of cartilage. Furthermore fragments of cartilage have been seen in normal synovial fluid (HAMMAR, 1892; KEY, 1928; BAUER, BENNETT, MARBLE, and CLAFLIN, 1930; EKHOLM and NORBÄCK, personal communication).

This wear has been considered to be especially pronounced in old and degeneratively changed articular cartilages since the synovial fluid of such joints contains more detritus than that of normal joints (BAUER, BENNETT, MARBLE, and CLAFLIN, 1930; HULTÉN and GELLERSTEDT, 1940).

HULTÉN and GELLERSTEDT (1940) assumed that the amount of the wear of a normal articular cartilage also varies with the degree of exertion on a joint. The correctness of this assumption has been confirmed in an experimental investigation on rabbits by EKHOLM and NORBÄCK (personal communication). In a special apparatus one knee-joint was exercised, whereas the other was kept in a state

of rest. A quantitative and qualitative examination of the formal elements of the synovia from both the knee-joints was carried out. It revealed that the functioning led to an increase of the products of wear and tear of the surface of the articular cartilage as well as that of the synovial membrane. The magnitude of this increase seemed to depend on the nature and duration of the exercise in such a way that an augmented loading and a longer duration resulted in a greater wear of the cartilage.

The other question which was of pertinence to the capacity of the cartilage to continuous regeneration, has been the object of wide interest. Apparently, however, the usual method of attaining an opinion of the regenerative capacity of cartilage seems to have been the study of the tendency and course of the repair of defects applied to cartilage. Such investigations have often led to divergent results. The lack of agreement seems to depend on such factors as different methods, the length of the time of observation, the position and depth of the defect within the articular cartilage; but also on the fact that a distinction was not always consistently made as to whether the results were obtained from articular cartilage or from cartilage covered with the perichondrium (costal cartilage, auricular cartilage).

Defects of the latter were filled with connective tissue growing from the perichondrium, and only in solitary cases did the cartilaginous tissue itself reveal a minute tendency towards proliferation (Gussenbauer, 1871; Tizzoni, 1878; Marchand, 1901; Matsuoka, 1904; Mori, 1905).

The defects localized near to the margin of an articular cartilage and those that penetrated through the cartilage to the subchondral marrow cavity were repaired in principally the same way. Such defects were filled with connective tissue growing from the attachment of the capsule and the marrow, respectively. Through metaplasia of the cells of this tissue and a varying degree of secondary hyalinization of the fibrous cicatrix, a more or less cartilaginoid tissue was obtained, which sometimes made up entirely for the loss of cartilage (Tizzoni, 1878; Gies, 1883; Seggel, 1904; Ciociola, 1921; Ito, 1924; Häbler, 1925; Shands, 1931; Bennet, Bauer, and Maddock, 1932).

If the defect, on the other hand, was made in the middle of the articular surface and did not penetrate into the marrow, the result would be different. In such cases, some workers have not been able to find any tendency towards a regeneration of the cartilage (GIES, 1883; CIOCIOLA, 1921; HÄBLER, 1925). Others, on the other hand, described their observations of a proliferation of the cartilage cells adjacent to the defect (TIZZONI, 1878; SEGGEL, 1904; FASOLI, 1905; FISHER, 1922—23; SHANDS, 1931; BENNET, BAUER, and MADDOCK, 1932). These proliferations of the cartilage, however, were not of such a magnitude as to make up for the defect. Moreover, BENNET, BAUER, and MADDOCK (1932) found that the tendency of regeneration was greater if the defect was localized within the weight-bearing part of the articular surface. This condition is ascribed by them to a stimulating effect of the functioning of the joint.

An observation made by FASOLI (1905) is especially worth mentioning. As a matter of fact, he found that *very small defects* of the articular cartilage healed completely by independent progressive proliferative formations of the cartilage on the sides of the defect, where he could also observe divisions of the cells.

This case seems to be of special interest as it may, to some degree, be compared with the very small defects arising in the surface of a joint in a physiological wear and tear of the cartilage.

The investigations related above have revealed that the capacity of cartilage to repair artificial defects is relatively limited. In all appearance, the results of these investigations have contributed to the view occasionally advanced that the articular cartilage scarcely possesses any capacity of regeneration whatever. From a biological point of view, however, a distinction must be made between such signs of regeneration as appear in connection with the repair of artificial defects and such a regenerative capacity of a normal articular cartilage as is necessary to make up for the physiological wear of the latter. This has also been pointed out by HULTÉN and GELLERSTEDT (1940).

The current opinion is that such a process of continuous regeneration must exist in a normal articular cartilage. From where this process originates, and how it proceeds, however, is little known. The various theories concerning these questions are based on isola-

ted observations in histological sections, but to a great extent also on assumptions.

BOEHM (1868) and WEICHSELBAUM (1877) consider that even the central parts of an articular surface regenerate by the cartilaginification of connective tissue growing from the margin of the articular cartilage. This opinion has already been refuted by HAMMAR (1892) as being hardly plausible. FISHER (1922—23, 1939) assumes that "the superficial cells of the articular cartilage are the source from which the deeper cells are derived." A third hypothesis is that the regeneration occurs in the parts of the cartilage located a short distance below the surface. This thought was first presented by OGSTON (1876), who spoke of a "focus of central growth." The location of this "focus" seems to agree with what is, in this work, designated the transitional zone. He is of the opinion that from this focus there is growth partly towards the bone and partly towards the surface of the articular cartilage. HAMMAR (1892) also supports the view that the regeneration takes place below the surface in the interior of the cartilage.

The majority of authors hold that the process of regeneration consists in a reproduction of the cellular as well as the intercellular components. No reliable proof as to the reproduction of the cells has been presented, since mitotic cell-division does not seem to occur in adult articular cartilage. HAMMAR (1892) and ELLIOT (1936), however, observed shapes of nuclei, which were interpreted as signs of amitotic cell-division. The location of such cells within the cartilage corresponded largely to that of the transitional zone and the deep part of the tangential zone. In foetuses and very young individuals *mitoses* have been observed within this very region (HARRIS, 1933; ELLIOT, 1936). ELLIOT is of the opinion that this supports the assumption that the above shapes of the nuclei are really signs of cell-division.

LUBOSCH (1910), PAYR (1918), and MÜLLER (1929), on the other hand, have given expression to the thought that the articular cartilage, with respect to its cellular component, is to be regarded as a permanent organ ("Dauerorgan"). The number of the most superficial cells, however, is assumed to be constant through amitotic divisions, and the intercellular substance to be continually trans-

formed in the interior of the cartilage as an adaptation to the stimulus caused by the tangential displacement of the cartilaginous tissue in the functioning of a joint ("Abscherungsbewegung").

Finally, it may be pointed out that several workers have assumed that the function of a joint has a stimulating effect on the processes of growth and regeneration in the articular cartilage (ROUX, 1895; FICK, 1904; BENNINGHOFF, 1925; BAUER, ROPES, and WAINE, 1940).

The mechanism of the formation of intercellular substance is not yet quite cleared up. A number of theories have been advanced. During the last decades experiments with tissue-cultures have contributed to the current view of our day. According to the latter, the process consists of a reciprocal action between the cells and the tissue-lymph surrounding these. The cells appear partly to contribute to the production of the chondromucoid, and partly to influence the precipitation of intercellular collagenous fibrils in the matrix. As these problems will not be taken up for discussion in the present investigation, there is no detailed account of them here. SCHAFFER (1930) and HOLMDAHL and INGELMARK (1948) have presented us with reviews of literature concerning this question.

The Problem

It appeared from the review of literature that there is an intimate connection between the function of a joint and the growth and differentiation of the articular cartilage in growing individuals; as well as between the function, on the one hand, and the architecture, nutrition, and resistance of an articular cartilage, on the other. Furthermore, the function of a joint normally causes a wear and tear of the surface of the articular cartilage. Yet it also seems to stimulate the regeneration of the cartilaginous tissue in the repair of defects in the articular cartilage.

However, the manner in which the tissue of a normal, adult articular cartilage reacts when influenced by a physiologically increased function is practically unknown. As far as I have been able to see, no experimental investigations have been carried out which can elucidate this question.

For this reason I found it justifiable to make an attempt at examining this problem. In so doing I endeavoured to prepare and carry out the present investigation in such a way that the following questions might, if possible, be elucidated, or answered.

1. Will the tissue of an adult articular cartilage show signs of quantitative changes relatable to an increased functioning of a joint?
2. If that is the case:
   a) Can they be observed in both the cellular and the intercellular component?
   b) Is such a reaction localized mainly to a certain zone of the articular cartilage, or does it occur more uniformly spread?
3. What effect has the duration of the increased functioning upon these changes? Will the different components of the tissue react simultaneously?
4. Finally, can the results obtained give us any information as to the course of the continuous regeneration of an adult articular cartilage?

Material and Methods

Material. Arrangement of Experiments.

In the present investigation there were used 140 adult, male guinea-pigs belonging to the same strain.

The experiments were started when the animals were 11 $^1/_2$ months old. On the one hand, the material was divided into two main groups in such a way that 60 animals were subjected to systematic exercise, whereas 80 served as control-animals. On the other hand, the material was divided into six different subgroups, the animals of which were killed after certain intervals. As to the number of animals in the groups, reference is made to Table 1.

When the material was to be divided into the two main groups, animals for control and those for exercise, their weights were taken as the basis for selection. By the guidance of these weights the animals were distributed in such a way that the average weight in each group was the same, as was the weight distribution.

The animals of the subgroups I—VI were not taken out from the two main groups until such a subgroup was to be killed. In so doing,

TABLE 1.

The Number of Animals in the Different Groups.

Groups	Number of Unexercised Animals		Number of Exercised Animals	
	Originally	Finally Examined	Originally	Finally Examined
I	20	19	—	—
II	10	9	10	10
III	10	8	10	10
IV	10	9	10	10
V	10	9	10	10
VI	20	19	20	17

the actual weights of the animals and the weight distribution within each group at that time were even now taken as the basis for selection. The intention was to have the selected subgroup reflect the main group as much as possible both with respect to its average weight and to the weight distribution.

A comparison between the average weights of the groups revealed no statistically significant difference between Groups I and VI of the control-animals, nor between control-animals and exercised animals.

The animals were exercised in an apparatus in which the floor was an endless band of canvas driven by an electric motor. The speed was kept constant, and so great that a point on this band covered 1,000 meters in 40 minutes.

Relatively soon the animals learnt to run in this apparatus. As soon as after 3—4 days the exercise intended could be carried out according to a definite schedule, i.e. the animals had to run 1,000 m each day. Two or three intermissions of 10—15 minutes each were introduced so as not to have the whole distance covered at the same time. By keeping the exercise of the animals at such a moderate level, it was expected to avoid their exertion with an eventually concomitant pathological reaction of their articular cartilage. After their daily exercise the animals used to rest for only about 10 minutes after which they moved about freely in their cages in at least the same degree as the control-animals.

At the same time as the regular exercise began, the animals of Group I were killed. The exercise was then continued with the above distance of 1,000 m per day. After 20 000, 40 000, 60 000, 80 000, and 100 000 m of exercise the animals of Groups II—VI were killed, and simultaneously with these the corresponding control-animals of the same groups.

Exercised as well as unexercised animals lived in cages having a floor-area of 40×50 cm, each cage holding five animals.

The feed of the animals consisted of oats, Swedish turnips, and hay, and in summer fresh grass.

(For further discussion, see p. 35.)

Histologic Technique.

The animals were killed by means of ether, after which their shoulder-joints were dissected out immediately. The humerus and the scapula were sawn off, by which their medullary cavities were opened. Connecting tissues were trimmed off from the articular capsule, which was otherwise left intact.

The joints were then fixed for 5 hrs. in Carnoy's mixture (60 cc absolute alcohol, 30 cc chloroform, 10 cc glacial acetic acid). After decalcification with a 5 per cent trichloro-acetic acid for 15 days they were embedded in paraffin.

Only one shoulder-joint from each animal was investigated. As a rule, the right side was chosen. In those cases where it was impossible to obtain histotechnically satisfactory sections from this side, the left joint was sectioned and examined. In some cases there was no possibility of getting a satisfactory section, either from the right joint or the left. These animals have not been included in the material.

Can it be considered sufficient for the purpose of the present investigation to examine the joint of only one side? It did not seem probable that the right side should be different from the left with respect to the factors under investigation. Nor did a comparison between the right and the left sides of the control-material reveal any differences as to the numbers and sizes of the cells. This result also agrees with the findings of HOLMDAHL and INGELMARK (1948) on growing rabbits, in which no differences between the right side and the left were found with regard to the structure of their articular cartilage.

In order to obtain comparable sections, it was necessary to take them from the same locality of the joint in all the animals. For this purpose, the following method was elaborated:

A sewing-needle 0.5 mm thick was inserted into the marrow cavity along the axillary border of the scapula (Margo axillaris) towards the middle of the glenoid cavity. After that, a determination was made of the position of the sagittal plane dividing the head of the humerus into a medial and a lateral half. This plane was then marked onto the tuberculum majus. A second needle was inserted into

the marrow cavity of the humerus-shaft from the sawed-off end of the bone towards the mark on the tuberculum majus, and care was observed to have this needle lying on the same plane as that in the scapula. The sagittal plane indicated by these two needles divided the joint (humerus and scapula) into a medial and a lateral half.

After the paraffin-embedding, the paraffin-block was cut so that a great part of each needle was visible. In the microtome the block was oriented in such a way that the plane of sectioning was parallel with that of the needles.

The medial half of the joint was cut off in rather thick sections. Immediately before the plane of sectioning coincided with that of the needles, the latter were taken away, and the sectioning was continued until the canals made by the needles were cut open. At this stage some sections 15 μ thick were taken for examination. All the sections used for this purpose were taken on the level of these canals, and stained with Ehrlich's haematoxylin and eosin.

One section from each animal was chosen for further investigations. In making this selection regard was only taken as to the histotechnic quality of the sections.

In order to make it possible to examine a corresponding locality within each section, the sections were mapped according to the principle introduced by HOLMDAHL and INGELMARK (1948).

In this case the method was carried out in the following way: The slide with the mounted section was placed on a protractor and

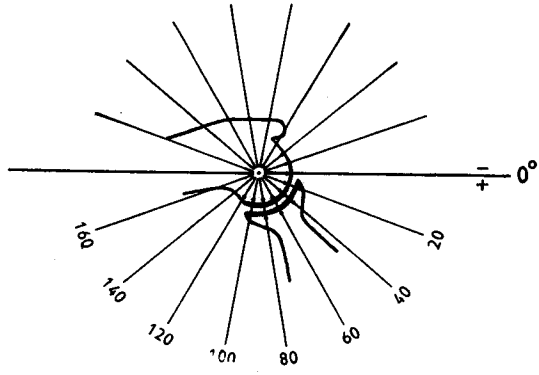

Fig. 1. Schematic drawing to illustrate the mapping of the sections.

oriented in such a way as to make the most proximal part of the external contour of the humerus parallel with the zero-line of the protractor, at the same time as the zero-point of the latter coincided with the approximate centre of the cartilaginous periphery. The centre and the zero-line as well as every 20th degree were marked on the coverglass with dots of India ink immediately below the limit between the cartilage and the bone belonging to the humerus and the scapula respectively (Fig. 1).

This method seems to present a satisfactorily uniform orientation in the sections when carried out as indicated and by the same person.

(For further discussion, see p. 36.)

The Counting of the Cells.

When the sections were examined microscopically, primarily for the purpose of registering the sizes of the cells and their number per unit volume, the three different zones of the uncalcified cartilage were treated separately. In the countings and measurements of the cells in this investigation use was made of the same magnification with a 60 × objective and a 10 × ocular.

An eye-piece-micrometer disk with a scale ("eye-piece micrometer") and an eye-piece-micrometer disk with 100 small squares in its central area ("eye-piece cytometer") were inserted together into the ocular. They were adjusted in such a way as to make it possible to observe both the scale and the squared area distinctly at the same time (Fig. 2). By this arrangement the cells of a certain zone could be counted within a chosen unit of volume, after which a certain number of the same cells could be measured without changing oculars. In the magnification used the length of one side of the eye-piece cytometer corresponded to 125 μ, and each division of the eye-piece-micrometer scale to 2.4 μ.

A rectangle (constituting a part of the eye-piece cytometer) consisting of ten small squares—in Fig. 2 marked with a heavy line—will from now on be called *one rectangle*. With regard to the different widths of the tangential, transitional, and radial zones, and the different densities of cells per unit volume in them, a certain mul-

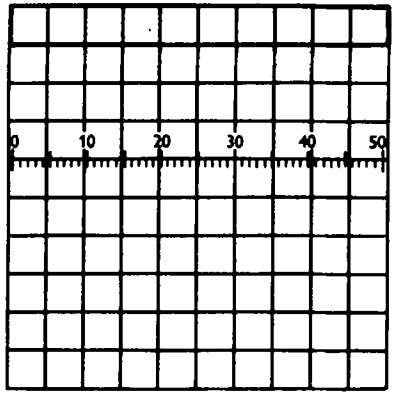

Fig. 2. The squared area of the eye-piece cytometer and the scale of the eye-piece micrometer as seen in the ocular.
The heavy line marks *one rectangle*.

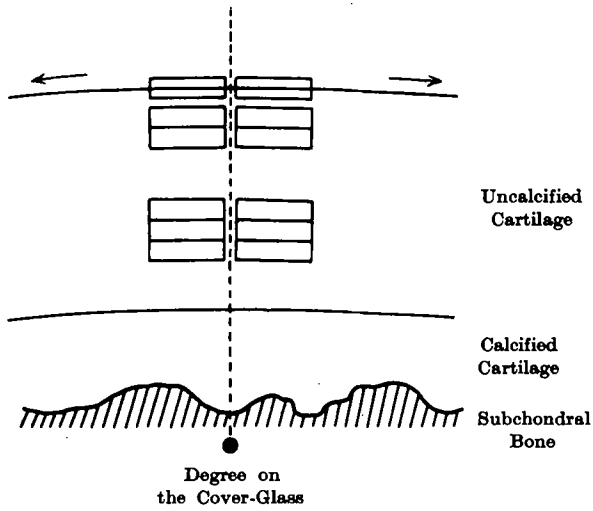

Fig. 3. The figure illustrates the position of the reference units of area in counting the cells of the different zones of the uncalcified cartilage.

tiple of such a rectangle was chosen for each zone as the reference unit of area within which the cells were counted. As seen in Fig. 3, the unit of area used was half a rectangle in the tangential zone,

two rectangles together in the transitional zone, and three rectangles together in the radial zone.

With +60° as a starting-point on the humerus, five units of area were placed successively towards the next lowest and next highest degrees respectively, as indicated by the arrows in Fig. 3. The length of the total area within which the cells were counted came in this way to be equal to the length of ten rectangles and approximately as great as the distance between two marked degrees. The distance between two successive rectangles corresponded to the diameter of one cell, thus making it possible to avoid counting any cell twice. The sections were examined systematically by raising and lowering the microscope tube.

The counting of the cells was carried out in accordance with the principle of counting blood-corpuscles. The examination of the scapular cartilage was made in the same way as that described for the humerus. For the scapula, however, the 10th degree which was located next to the middle of the scapular cartilage was chosen as a starting-point for the examination.

(For further discussion, see p. 39.)

Determination of the Number of Cells per Chondron.

At the same time as the cells were counted within the different zones, the number of cells per chondron was also registered. In so doing the chondrons were classified according to their number of cells, viz. 2, 3, 4, etc. In order that two or more cells might be included in the same chondron, it was essential that they should lie contiguous to each other without any intercellular substance separating them. A registration was made of each chondron that had such a location within the zone that at least one of its cells belonged to the area within which the counting of cells had been carried out.

(For further discussion, see p. 41.)

The Measurement of the Cells.

In connection with the counting of the cells within the various zones a determination was made of the lengths of the axes of six

cells in every other unit of volume counted, i.e. a total number of 30 cells in each zone. In so doing the cells were always measured in a predetermined sequence, the possibility of selecting special cells thus being excluded. Only cells apparently deformed, and those covering each other in such a way as not to be delimited with certainty, were excluded.

The shape of the cells in this sagittal section was approximately elliptical or circular. The lengths of the two cell-axes measurable in this section were determined by means of the eye-piece micrometer. At first, a measurement was made of the axis most nearly parallel with the articular surface ("the 1st cell-axis"), after which the ocular was turned 90°, and the length of the axis perpendicular to the former one was determined ("the 2nd cell-axis").

By raising and lowering the tube of the microscope, the focal plane exposing the largest axes of each cell was found. If the cell was retracted in the cell-cavity, the latter was measured instead.

In order to calculate the volumes of the cells, it is necessary to know the dimension of the cells, in the direction perpendicular to the sagittal plane ("the 3rd cell-axis"). It would have been desirable to have a measure of this cell-axis from each zone of cartilage of the humerus and the scapula, as well as from every individual animal. The preparation and examination of the sections necessary for this purpose proved to consume so much time that these intentions could not be realized. In order to obtain some information about this quantity measurements were made on four animals—one from Group I, one from the exercised animals in Group II, one exercised and one unexercised animal from Group VI.

The lateral half of the joint remaining after the sagittal sectioning was used for this purpose. The humerus and the scapula were separated and treated individually. The problem was to construct a sectioning-plane lying perpendicular to the sagittal plane used earlier and meeting the articular cartilage tangentially at point $+60°$ on the humerus and at the middle of the scapular cartilage, respectively. This was made possible by means of four needles so connected that they formed two planes at right angles with each other. The part of the paraffin-block containing the humerus was trimmed to make the convexity of the humeral head visible, as is indi-

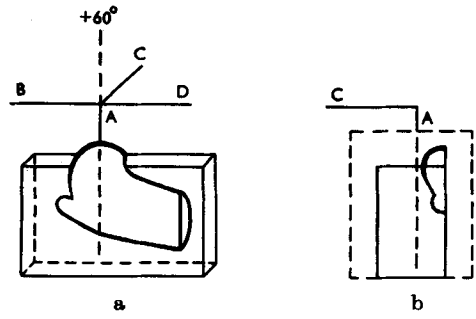

Fig. 4. The re-embedding of the humerus for tangential sectioning.
a. Orientation of the needle-plane DBC parallel with the tangent to the articular cartilage at + 60°.
b. The preparation as seen in the direction B—D after being re-embedded. The new paraffin-block marked by the dotted line.

cated in the illustration (Fig. 4 a). One of the needles (A) was inserted into the block abreast point +60° (marked on the preparation with a line running through +60° and the approx. centre of the humeral head) and, moreover, having a direction parallel to the sagittal plane. Then the three other needles formed a plane at right angles with the sagittal plane used earlier. The whole preparation was embedded into a new block, three needles however being left visible, as shown in Fig. 4 b. These three needles served as a guidance when the new sectioning-plane was adjusted in the microtome.

The scapula was treated principally in the same way. In this case, needle A was inserted into the paraffin-block abreast the middle of the scapular cartilage, being at the same time adjusted parallel to the sagittal plane used earlier. This new sectioning-plane, BCD, thus came to lie parallel with the tangent to the middle of the glenoid cavity.

The cartilages were sectioned into serial sections 5 μ thick, which were stained with Ehrlich's haematoxylin and eosin.

The appearance of these tangential sections of the humerus resembled the segment of a circle where the chord corresponded to the sagittal plane of sectioning. The sections of the scapula had the same appearance as soon as the sectioning had attained such a depth that the middle of the glenoid cavity was included in the section.

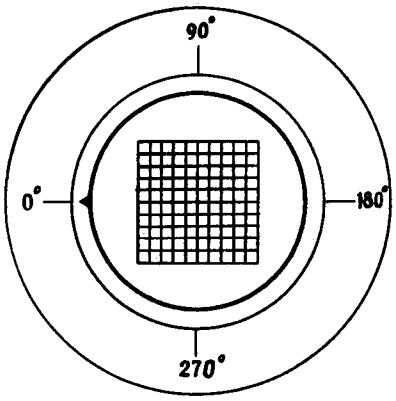

Fig. 5. Schematic drawing of the goniometer. The pointer is turned to 0°, one side of the squared area being parallel with the 0°—180° line.

For the examination of the tangential sections, the microscope was equipped with a goniometer, the protractor of which was fixed onto the tube of the microscope, and the pointer onto the ocular. The pointer of the goniometer was adjusted in such a way that, when it pointed to 0°, one side of the eye-piece cytometer was parallel with line 0°—180° of the protractor, and consequently the other side with line 90°—270° (Fig. 5).

When the section was oriented into the field, a control was first made as to whether the pointer had the position of 0°. The stage was then revolved so as to make the chord of the section coincide with the side of the eye-piece cytometer corresponding to line 90°—270°. Hence the direction perpendicular to the chord agreed with line 0°—180°. This arrangement made it possible to orientate the cell-axes of the tangential sections in relation to the sagittal plane of sectioning.

The axis lying within the angle $\pm$ 45° was designated as the 3rd cell-axis. A measurement was made of this axis in 20 cells from every section. The examination started with the sections corresponding to the tangential zone, and then continued deeper down into the cartilage even to approximately the middle of the radial zone. About 20—25 tangential sections of each cartilage were examined.

The cells were measured within a very limited region next to the

chord. By comparing characteristic structures recurring in adjacent sections it was always possible to examine a region having the same position in each section.

By this procedure, each region examined came to lie deeper in the articular cartilage than that of the preceding section. This would not have been the case if, for instance, the region examined had been next to the chord, in one section, and relatively closer to the arc of the segment, in the following one.

By measuring the width of each zone of the cartilage in the sagittal section it was possible to make an approximate determination of the number of tangential sections belonging to the different zones respectively.

In examining the tangential sections, the axis perpendicular to the 3rd cell-axis was also measured, as well as the length of the projection of the cells upon the sagittal sectioning-plane. Moreover, as to each cell measured, a registration was made of the angle between its longest axis and the sagittal sectioning-plane.

(For further discussion, see p. 43.)

Computing the Volume of the Cells.

The cell-axes obtained from each zone of the sagittal sections (the 1st and 2nd axes) were compared with those of the tangential sections (the 3rd axis) from the same humerus and scapula. This comparison revealed that the 1st cell-axis could be considered equal to the 3rd. When this fact was connected with the shape of the cells in the sagittal sections it seemed possible to regard them as revolution-ellipsoids. Thus the rotation was considered to occur around the 2nd axis.

The volume of each individual cell was computed according to the formula of a revolution-ellipsoid, $\frac{4}{3}\pi a^2 b$, where "b" was half of the 2nd cell-axis around which the rotation was considered as occurring, and "a" half of the 1st cell-axis. The volumes thus obtained from each individual cell were added to each other, and the average volume of the cells was computed.

In the tangential zone the cells, as a rule, had the form of oblate

spheroids. The cells of the transitional zone were less flattened, being often spheroids, and in the radial zone usually prolate spheroids.

(For further discussion, see p. 44.)

Determination of the Thickness of the Uncalcified Cartilage.

As the above analysis only dealt with the uncalcified cartilage, it was of essential interest to obtain a definite conception of its thickness. The measurement of this was carried out within the weight-bearing part of the articular surface at the point from which the counting and measurement of the cells were started.

It was not possible in all the animals to distinguish a distinct limit between calcified and uncalcified cartilage by means of the microscope. In approximately 75 per cent of them such a limit could be distinguished by using blue-glasses of various densities and varying the opening of the diaphragm.

As to the remaining 25 per cent, however, it proved to be possible to distinguish between the two layers rather well by means of another procedure. Within the most basal parts of the articular cartilage (those corresponding to the position of the calcified layer), the cells were larger and lighter in colour than within the part of the cartilage corresponding to the radial zone, having as a rule small pychnotic nuclei. By following the cartilage along a certain length it was also possible to ascertain that the columns having cells typical of the radial zone and the calcified cartilage, respectively, met each other within a rather evenly running and narrow belt. This gave the impression of corresponding to the position of the limit between the calcified and the uncalcified cartilage. These observations were compared with those of sections where a limit could be distinguished. It was then observed that there was good agreement between the position of the belt described and the visible limit.

In order to obtain as objective measurements of the thickness of the cartilage as possible, the slides were supplied with ciphered labels so that the examiner could not recognize the groups to which the different animals belonged.

The measurements were made by means of an eye-piece micrometer having 100 scale-divisions. Each division corresponded to 7.8 μ. In measuring there was no difficulty in reading semidivisions.

When there was a visible limit, the distance between this and the articular surface was of course measured. When no limit could be distinguished, the measurement was started from a limit approximately obtained within "the narrow belt" described.

The measurements were always carried out at right angles to the articular surface. In order to control this more accurately, a fibre of glass-wool was fixed to the eye-piece-micrometer disk perpendicular to the scale.

(For further discussion, see p. 46.)

The Determination of the Thickness of the Sections.

It is known that the thickness of the sections obtained from a microtome varies in spite of the latter being adjusted to the same thickness during the entire sectioning. In our case this implies that various animals might be represented by sections having different thicknesses. As such a condition would necessarily influence the number of cells registered per unit volume, it was decided to measure the thickness of each section employed.

This was determined by means of a microscope supplied with a graduated micrometer-screw. The procedure was carried out in such a manner that a structure of the upper surface of the section and one in the lower was focused, and the difference between the two foci was read on the micrometer-screw.

(For further discussion, see p. 47.)

The Statistical Treatment of the Material.

At first the groups of the control-material were compared with each other as to the number of cells, the volume of the cells, the thickness of the cartilage, and the number of cells per chondron. The differences observed could be ascribed to random variations. For this reason the control-animals were conjoined into a single group.

Each exercised group was then compared with the control-material. Since the number of animals in the exercised Groups II—VI was small, the method indicated by FISHER (1948) was followed in calculating the standard error of the difference.

According to DAHLBERG (1940), differences exceeding 3 times their standard error were regarded as statistically significant, and differences $2\,{}^1/_2$—3 times their standard error as statistically very probable.

The accuracy of each method (the error of method) employed (σ_i) has been calculated from the differences between double determinations, according to the formula given by DAHLBERG (1940):

$$\sigma_i = \sqrt{\frac{\Sigma d^2}{2n}}$$

d = the difference between two determinations.

n = the number of double determinations.

The error of method was expressed in percentages of the mean and the standard deviation, respectively. The magnitude of the error of the different methods is given in the corresponding parts of the following chapter.

Discussion of the Material and the Methods

Concerning the Material.

(For its presentation, see p. 21.)

The essential demands on the material of an investigation of this kind are that it is quantitatively sufficient enough to allow a statistical treatment of the findings registered, and that it is as far as possible uniform.

Since the stock of these animals in Sweden was decimated during the course of World War II, certain difficulties were faced in fulfilling these conditions simultaneously.

It would of course have been especially valuable from a statistical point of view if the different groups had consisted of a greater number of animals. It was, however, impossible to make this so without diminishing the demands on the uniformity of the material. Moreover, an additional increase of the material would have made this time-consuming investigation too laborious.

Unfortunately it was not possible to obtain utilizable sections from all the animals. The number of animals finally examined is seen in Table 1.

All the guinea-pigs used in the present investigation belonged to the same strain originating in 1934 from twenty animals.

Males were exclusively used in order to avoid the results being influenced by eventual divergences in constitution and the manner of reaction of the animals depending upon sex.

As indicated above, the present investigation was carried out in order to obtain a conception of the manner of reaction of the articular cartilage in adult animals. According to Raebiger (1923), guinea-pigs are adult at an age of 8—9 months. Jaffé (1931) states that the growth of guinea-pigs in the sense of "the final develop-

ment" of their skeletons attains its completion at approximately eight months of age.

At the beginning of the experiment all the animals were 11 $^1/_2$ months old, with a variation of $\pm$ 1 week.

A microscopical examination of the sections of the 1st control group revealed the fact that there was only a narrow remainder of the epiphysial cartilage of the humerus. This remnant was partly calcified and had no connection with the margin of the articular cartilage. The growth zone was closed.

These facts seem to justify the assertion that the animals can be regarded as adults, and that the material is uniform also from the viewpoint of their age.

The importance of a uniform material is stressed by SILBERBERG and SILBERBERG (1942). They point out that the normal time-curve of "skeletal development proceeds at a faster rate in females than in males, in breeding females more rapidly than in virgins;" and in certain strains more rapidly than in others.

Discussion of the Histotechnical Procedure.

(For this procedure, see p. 23.)

Histotechnical treatment always changes a tissue more or less. The quantitative morphological analysis of the tissue is consequently always connected with several sources of error, and unfortunately it is very difficult to determine the greatness of each of them. For this reason it is urgent that the histotechnical procedure is carried out with such an accuracy that these inevitable sources of error will not be greater than necessary. In addition, it should be seen to it that the entire material is uniformly dealt with.

The animals were not killed until the day after a given exercise, because it might have been possible that this exercise had brought about temporary changes in their articular cartilage. Such changes, in their turn, might have influenced the results. Subsequent investigations have proved that this precaution was justified. Compare what has been said concerning the flow of fluid through the articular cartilage and its increase in thickness in immediate connection with

an increase of function (INGELMARK and SÄÄF, 1948; INGELMARK and EKHOLM, 1948 and personal communication). (See p. 12.)

After killing the animals their shoulder-joints were dissected out so rapidly that these could be put into fixing-fluid within ten minutes. In so doing the preparations did not dry before being fixed, which would have brought about a greater shrinkage (TARKHAN, 1931).

All changes of fluids in the histotechnical procedure were made at exact points of time. The preparations were transferred direct from one fluid to the next, thus preventing their being dried in connection with the changes of fluids, which would also have increased their shrinkage (TARKHAN, 1931).

The fixation fluid, Carnoy's mixture, has an excellent capacity of penetration, fixes the tissues well (TELLYESNICZKY, 1898; SEKI, 1937), and can advantageously be utilized for cartilage (RUPPRICHT, 1910). Its shrinking effect is generally considered to be slight, and in the case of tendons it amounted to 1 per cent (SEKI, 1937).

The dehydration and paraffin-embedding following a fixation is regarded as causing a great shrinkage (TARKHAN, 1931; SEKI, 1937; STOWELL, 1941). How great it was in the case at hand is not known.

Until they had been embedded in the paraffin-block all the preparations were treated uniformly. But when sectioned and stretched, each individual section was the object of manual treatment, when one must take into account the fact that every section will not be treated exactly alike, in spite of deliberate care to this effect. As indicated above, there was moreover a variation, due to the microtome, in the thicknesses of the sections. This source of error was diminished by determining the thickness of each section, and then correcting the number of cells registered with respect to this thickness.

Another source of error may also be connected with the variation of the thickness of the sections. According to certain observers, thin sections reveal a greater tendency towards distortion than thicker ones (AUMONIER, 1938; STOWELL, 1941). In order to control whether this source of error was of importance to the articular cartilages in this investigation, a joint was cut in serial sections alternating between 15 μ and 10 μ. A determination was made of the humeral cartilage of 20 sections of either of these two thicknesses in such a

manner that their cartilages were drawn in a drawing apparatus at 30 × linear magnification, after which their sizes were determined by means of a planimeter. When the two series were compared, no difference was observed.

In the stretching and drying of the sections it is essential to see to it that they are not exposed to temperatures exceeding the melting-point of the paraffin used, since the distortion and shrinkage of tissues will then increase (SUGITA, 1917). In the present investigation, it was in some instances observed that the paraffin on the slide had melted. Such sections were discarded.

All the sections were stained in the same way, and every period of this procedure always had the same length.

By these discussions, some factors changing the volume of tissues during histotechnic procedure have been pointed out. We have seen that there are many factors which more or less affect a tissue. As long as the preparations are treated exactly alike, the greatness of a source of error is not so important, provided that the different articular cartilages have also reacted similarly during the procedure. Those phases of the method where each section is the object of manual and individual treatment no doubt present the most outstanding points of uncertainty.

It would of course have been of great value if we had exactly known in what direction each phase of the histologic technique had influenced the tissue as well as the magnitudes of these sources of error with respect to the tissue at hand. Unfortunately, it has been impossible to carry out such a systematic control. But in view of the following factors:

that the entire material has been treated as uniformly as possible;
that, in the studies of the sections, nothing has been revealed, and, furthermore, nothing is known which can give us a reason to assume that the various groups had reacted in a different way on the histotechnic treatment; and
that there was no intention to obtain absolute numbers, but only such as allow comparisons between the various groups;

it would in any case be correct to regard the sections produced as utilizable.

However, in addition to what has been said above, a fact will here be adduced that might be of importance when comparing histotechnically treated material from exercised and unexercised animals, respectively. HOLMDAHL and INGELMARK (1948) indicated that qualitative differences of a tissue in life may be transformed into quantitative differences in connection with the histotechnical treatment of the tissue.

In support of this view there is an interesting observation by INGELMARK (1948) in an electron-microscopic investigation into the calibres of collagenous fibrils. If the measurements were carried out on fresh tissue, there appeared no differences between exercised and unexercised individuals; but if the tissue had been impregnated with osmium tetroxide prior to the measurements, there was a statistically significant difference between the two groups. Whether such a condition exists concerning unexercised and exercised animals of the present investigation is quite unknown.

Discussion of the Method of Cell-Counting.

(For this method, see p. 25.)

An eventual reaction in the articular cartilage in connection with an increased functioning of a joint could very likely be expected primarily within the weight-bearing part of the articular cartilage. To determine definitely where the limit between the weight-bearing and the non-weight-bearing part of the articular cartilage runs, seems to be of subordinate significance. Nevertheless, it is necessary to make sure that the region which is the object of further examination is actually located within the weight-bearing part of the articular cartilage.

By the fluoroscopy of living guinea-pigs and through observations when dissecting out the joints, the conception was obtained that, in the sagittal sectioning-plane used, the points of the articular cartilage corresponding to + 60° on the humerus, and the middle of the scapular cartilage, fulfilled the requirements mentioned, and could most suitably be chosen as starting-points for the examination.

As pointed out above, the three different zones of the uncalcified

articular cartilage were examined separately. In order to do this, however, it was essential to be able to make a distinction between the various zones to a certain degree of accuracy. Even if no exact border lines could be drawn between the different zones, yet it was feasible, without any hesitation, to place the rectangles into each zone. The tangential zone holds a unique position in some respects. Due to the narrowness of this zone it has not been possible to make use of a broader unit of area in counting the cells than half a rectangle. In spite of this, it was necessary to have one margin of the area coincident with the free edge of the section. The narrowness of the tangential zone caused that cells lying in the outskirts of the transitional zone could occasionally touch the area in the tangential zone, whence these cells came to be included in the latter. Some uncertainty was also connected with the counting of the cells in this zone because the free edge of the section could sometimes be seen as slightly bending up and down alternatingly. This gave the impression of an undulation along the edge of the tangential zone. In such a case this bending off makes one count the cells within a larger area than when the entire cartilage lies on the same plane. Consequently the very same conditions do not seem to apply in counting the cells of the tangential zone and in the two others. These facts should be kept in mind when comparing the results of the various zones with each other.

The error of the method was determined in each of the three different zones of the humerus and the scapula, and in each zone 16 double determinations were made. In spite of the variations in the number of cells per unit volume between the different zones, however, the error of method, σ_i, showed a relatively small variation. For this reason the mean of the error of method of the different zones is given here.

	The error of method, σ_i:	σ_i in percentage of the mean:	σ_i in percentage of the standard deviation:
Humerus.....	2.3	2.7	15.2
Scapula.....	2.6	3.4	18.0

The number of cells registered from the sections, however, was not quite the same as the real number of cells per unit of volume.

As a matter of fact, since a number of cells were divided in the sectioning, parts of one and the same cell were to be found in more than one section. This fact resulted in the number of cells registered being too high. FLODÉRUS (1944), however, has elaborated a formula according to which it is possible to correct this error. Another factor of importance in this connection was the varying thicknesses of the sections. But since the thickness of each section was determined, and, moreover, as this thickness is included in FLODÉRUS' formula, it was possible to correct the number of cells with respect to both these sources of error at the same time. The number of cells registered in each zone was corrected so as to correspond to that of a section 15 μ thick.

FLODÉRUS' formula can be written in this case:

$$N = n \cdot \frac{15}{a + d - 2h}$$

N = the number of cells whose centres are located within a section 15 μ thick.
n = the number of cells and cell-fragments registered within the zone.
a = the thickness of the section in μ.
d = the arithmetical mean of the length of the 1st cell-axis in μ.
h = the height in μ of the thinnest cell-calottes included in the counting of the cells.

As the cells were regarded as revolution-ellipsoids, h was computed in the various groups according to the method indicated by KJELLGREN (1944). As to the humerus, h was practically 1 μ, whereas the scapula showed a somewhat lower value, viz., 0.8 μ.

Discussion of the Determination of the Number of Cells per Chondron.

(For this method, see p. 27.)

In such cases as where the cells were on different levels within the section, it was difficult to determine whether any intercellular substance separated them or not. For this reason cells in reality

separated from each other may have come to be counted as belonging to the same chondron. The registered numbers of chondrons may accordingly be too great. This error, however, applies to all the groups.

As appears from the description of the method, a number of the chondrons had one or more of their cells located outside the unit of area used in counting the cells. For this reason it was not possible to calculate the number of chondrons per unit volume. However, since the chondrons had always been registered in the same way, the numbers obtained could be used in drawing comparisons between the various groups. Yet in this connection it should be mentioned that the method of registration brought about an over-representation of the chondrons that had a large number of cells. On the other hand, HOLMDAHL and INGELMARK (1948) pointed out that chondrons with a small number of cells are over-represented in a histological section. For a large chondron has a greater chance of being cut by the sectioning-plane than a small one. For these reasons it is not possible to determine whether the chondrons registered present a true picture of their frequency within an intact cartilage.

As to the registration of the chondrons within the tangential zone[1], there was the same uncertainty as in the counting of the cells within this zone, though more pronounced. This is a consequence of the narrowness of this zone, for the chondrons were often partly in the outskirts of the transitional zone, but were represented as being only in the tangential zone.

The error of the method was computed from 20 double determinations of the number of chondrons in the transitional zone of the humeral cartilage. $\sigma_i = 2.4$; σ_i in percentage of the mean = 5.8 per cent; σ_i in percentage of the standard deviation = 27.3 per cent.

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[1] BENNINGHOFF indicates that, in a strict sense of the term, a chondron does not occur in the tangential zone. The designation is here used for practical reasons.

Discussion of the Method of Measuring the Cells.

(For this method, see p. 27.)

In measuring the cells an objective 60 × and an eye-piece 10 × were used. In this magnification one division of the eye-piece micrometer corresponded to 2.4 μ. The reading had an accuracy of $^1/_2$ a division. The error of method computed from 50 double determinations was: $\sigma_i = 0.38$; σ_i in percentage of the mean = 4.2 per cent; σ_i in percentage of the standard deviation = 14.5 per cent.

It may rightly be objected that the magnification might have been preferably greater in measuring the diameters of the cells. There was, however, another factor to be taken into consideration in this connection. As the three different zones of the uncalcified cartilage were examined separately with regard to the number and sizes of their cells, it was necessary to make an incessant control upon one's position in the cartilage. If use had been made of exceedingly large magnifications, such an orientation would have been considerably more difficult. Of course, by repeatedly alternating between small and large magnifications, it would have been possible to convince oneself of the fact that each cell to be measured *did* lie within the zone intended. This procedure, however, would have been too laborious.

For this reason a magnification was chosen that could be used simultaneously both in counting the cells and measuring them. By placing the eye-piece cytometer and the eye-piece micrometer in the same ocular, it was easier to make a continuous check as to whether the cells measured belonged to those counted.

Unfortunately, it was not possible, when measuring, to exclude a cell such as one that did not have its real axes within the section. Hence the fact had to be taken into account also that a number of cell-calottes had been measured, whose basal diameters had consequently been taken for the axes of a cell. The result of this was that the average volume of the cells was somewhat less than if the cell-calottes could have been excluded. But also in this respect there was a uniform examination of the material. Accordingly, there is no reason to assume that this source of error would upset the result of comparing the cell-volume between, for instance, the various groups.

Discussion of the Method of Computing the Volume of the Cells.

(For this method, see p. 31.)

When the volumes of the cells were computed, the latter were regarded as revolution-ellipsoids. It must be pointed out, however, that this way of considering the matter implies a schematization. But a study of the cells in various planes of sectioning revealed that, from a practical point of view, it was most correct to look upon the cells in this way.

A complete analysis of the method computing the cell-volume would have required a tangential sectioning of a great number of joints. As mentioned before this was not practicable, so one had to be satisfied with forming a general opinion of the shapes of these cells by means of a tangential sectioning of a few joints. The total number of observations within the tangential sections was exceedingly great; but, since the number of the joints examined in this way was as small as four, it has not been considered suitable here to occupy space by recording the figures obtained. Instead they are used to form the background of some discussion about this method of computing the volumes of the cells.

An attempt to analyse this method shows that there are rather a great many factors that may be thought of as having influenced the conception regarding the shapes of the cells and the computation of their volumes.

In the first place, the re-embedding of the preparation preceding the tangential sectioning may be held to have further affected the shrinkage of the cartilaginous tissue. Especially as far as the superficial parts of the cartilage are concerned, certain evidence of this shrinkage has been obtained from a comparison between the length of the 1st cell-axis and the projection of the cells of the tangential section upon the sagittal plane.

Another histotechnic factor to be stressed in this connection is that the tangential sections were only 5 μ thick. This may have caused the cell-calottes measured to have obtained another frequency than in the sagittal sections 15 μ thick. Especially as the tangential sectioning had cut the collagenic structure of the car-

tilage in another direction than that in which the sagittal sectioning had done, the thin tangential sections may, when stretched, have undergone changes, which in their turn eventually influenced the sizes and shapes of the cells. As to the direction and degree in which these factors have conjointly influenced the determination of the volumes of the cells, is a problem which at present eludes our powers of reliable appraisement.

The shapes of the cells in the sagittal sections were mostly similar to ellipses and circles respectively, though perhaps, only in comparatively few cases within each zone, they completely coincided with the mathematical requirements of these geometrical figures. As far as could be seen, this schematization did not affect the average volume of the cells in any special direction, as it resulted in an alternation of too high or too low values of the individual cells, and was moreover the same in the different groups.

The 3rd cell-axis could not be measured on the same cell as the 1st and the 2nd. On the other hand, an estimation of this axis was obtained by measuring tangential-section-cells located near the sagittal section examined. The mean of the 3rd cell-axis was compared with that of each of the two cell-axes measured in the sagittal section. This comparison was made within every zone of the uncalcified part of each cartilage. Of the 24 zones examined there were 17 that showed no difference between the 1st and the 3rd axis. In the other seven zones where a comparison revealed a difference in length between the 1st and the 3rd cell-axis, the 1st axis was longer in three cases, and the 3rd in four. This agreement between the mean of the two cell-axes was a reason for regarding the cells as revolution-ellipsoids. It does not imply, however, that every individual cell is a revolution-ellipsoid.

A study of the tangential sections, however, proved that a large number of their cells were round, or approximately round, and could consequently be regarded as separate revolution-ellipsoids where the rotation occurred around the 2nd cell-axis. Besides this, it was seen that the cells which were not round in the tangential sections as a rule had their long axis largely parallel with ($\pm$ 20°) the direction of the sagittal sectioning, or at right angles to it. This fact was of special importance, as it implied that the sagittal plane

of sectioning cut the cells parallel to one of their axes to be found in the tangential sections.

Such an orientation of the cells may be thought of as resulting in too high or too low values of the average volume of the cells when the "revolution-ellipsoid method" is applied. This fact appears, among other things, to be connected with how great the number of cells is that have their longest axis parallel with the sagittal plane of sectioning, but also with the proportion between the lengths of the axes measured in the tangential sections.

For the purpose of obtaining additional knowledge as to the reliability of this method of computation, the following reckoning was carried out: The 1st cell-axes were combined at random with the 3rd cell-axes. Sixteen series of combinations each having 30 combinations chosen at random were carried out in every zone of such articular cartilages as had been sectioned tangentially. The volume of each "cell" was computed according to the formula of an ellipsoid, regarding the 2nd axis as being constant. This cell-volume of each zone was compared with the volume obtained when the cells were regarded instead as revolution-ellipsoids. These different procedures of computation resulted throughout in practically the same values as far as the scapula was concerned, whereas in the humerus the revolution-ellipsoids presented a somewhat larger volume than the combinations made above.

Making a reservation for the effect histological technique may have had on the shapes and sizes of the cells, however, it appears to us as if the study of cells in various directions of sectioning has proved that from a practical point of view it is justifiable to regard them as revolution-ellipsoids.

Discussion of the Method of Determining the Thickness of the Cartilage.

(For this method, see p. 32.)

The accuracy of this method was determined partly with respect to the articular cartilages having a visible limit between the calcified and the uncalcified layer, and partly with regard to those where such a limit could not be distinguished. As to cartilages having vis-

ible limits as well as those without, 16 double determinations were made in the humerus and the scapula respectively. As the error of method, σ_i, was practically equal in the humerus and the scapula, the mean of their error of method is given here.

	The error of method, σ_i:	σ_i in percentage of the mean:	σ_i in percentage of the standard deviation:
Cartilages with a visible limit	3.3	1.5	8.1
Cartilages without a visible limit	8.6	3.6	17.7

The last figures indicate that even in measurements of articular cartilages without a visible limit, the assumed limit between the calcified and the uncalcified cartilage was, through the above method of procedure, placed in approximately the same position in the two observations.

It is of course possible, however, that the assumed limit was systematically placed in a wrong position. This would have resulted in the fact that the thicknesses of these cartilages would have become throughout either too thin or too thick when compared with that of the cartilages having a visible limit. The very agreement mentioned above between the location of the cell-columns in question and the position of a visible limit contradicted the possibility of any such appreciable systematic misplacement of the limit assumed.

By a comparison, in the control-material, between the articular cartilages having a visible limit and those without, no difference was to be seen as to the thickness of the uncalcified cartilage, whether in the humerus or the scapula. As the exercised animals were divided into small groups of various lengths of exercise, a corresponding comparison could not be made concerning this part of the material.

Discussion of the Method of Determining the Thickness of the Histological Sections.

(For this method, see p. 33.)

A more detailed account of this part of my investigation will be given in a special publication. However, the most important fac-

tors influencing the results of these measurements will be mentioned here.

As the thicknesses of the sections were determined by measuring the distance between a structure in the upper surface of the section and one in the lower, it was necessary to make a careful control that the structures which had been chosen should, as far as could be ascertained, represent the levels of these surfaces. Furthermore, it was essential that these two structures should be near the centre of the field.

An exact measurement of the thickness of a section would among other things have required that the upper surface of the section and the lower one, respectively, could have been reproduced in an actual image plane. On account of the depth of focus this was not feasible. By carefully controlling that the reading was always made at the very moment the first structure of the surface was distinctly visible, it was hoped that this error could be kept relatively constant. In all these measurements the same optical system was used (objective 40 ×, NA = 0.65, and eye-piece 10 ×).

Another factor which can influence the results of such measurements to rather a great extent, is the accommodational power of the eye. At first, the measurements of the thickness of each section were made when the eye had a normal power of accommodation. In so doing, it was revealed that the measure of the thickness of one and the same section could vary considerably owing to the degree of tiredness of the eye. Consequently, it was by no means certain that the degree of the accommodation was always the same. These observations were in complete agreement with those of JOHN's (1929—30).

A new series of measurements of the thicknesses of all the sections was then carried out; but, in order to reduce the above source of error as much as possible, the accommodational power of the eye was kept paralyzed by means of atropine during all these measurements. The value of the thickness of each section obtained in this way was used for correcting the number of cells registered.

In such conditions, the accuracy of the very measurements was determined by 36 double determinations. The error of method, σ_i = 0.05; σ_i in percentage of the mean = 0.4 per cent; σ_i in percentage

of the standard deviation = 3.6 per cent. The thicknesses of the sections in the double determinations varied between 9 μ and 14.5 μ.

The objection can of course be made that the variations in thickness found in stained and mounted sections from different animals may have arisen during the part of the histotechnical procedure following the sectioning.

In order to obtain a resolution of this problem, a serial sectioning of a joint was carried out. All the sections were taken on the level of the needle-canals, and dried and stained conjointly. A determination was made of the thicknesses of 28 serial sections as well as the number of cells per unit volume in the transitional and the radial zone.

The connection between the thickness of sections and the number of cells was examined by calculating two correlation coefficients—partly between the thickness of sections and the number of cells of the transitional zone, partly between those of the radial zone. These correlation coefficients were $+0.95 \pm 0.02$ and $+0.86 \pm 0.05$, respectively. The connection is, as obvious, unusually strong and speaks definitely in favour of the fact that the variation of the thicknesses of the sections had mainly arisen in the sectioning.

The method used in determining the thickness of the sections has several sources of error difficult to correct. In the present investigation, however, definite attempts have been made to diminish them and keep them under control. Due to the effect of the sources of error, it has presumably been possible to obtain only relative measures of the thicknesses of the sections. The strong connection between the thickness of sections and the number of cells that appeared in the serial sections proves, however, that the measurements were justified. It seems also to be reasonable to draw the conclusion that the values of the number of cells per unit volume will be more real if they are corrected with respect to the thicknesses of the sections.

Results

The following account will mainly be limited to results illustrating the reaction of the articular cartilage in connection with an increased functioning of a joint.

The results obtained from the statistical treatment could also have served as a basis for discussions of other facts of interest, as, for instance, differences existing between the zones of the cartilage with respect to their number of cells per unit volume, the average volume of the cells, and the volume of cellular substance per unit volume of tissue. As these questions are not of immediate pertinence to the actual problem, they will not be dealt with so much in this work. Some information, however, can be obtained from Table 16, p. 80, where a series of differences between the various zones of the cartilage as well as between the humerus and the scapula is to be found.

In Tables 13, 14, and 15, p. 78—79, there are given means and standard deviations of the number of cells per unit volume, the average volume of the cells, the volume of cellular substance per unit volume of tissue and the thickness of the uncalcified cartilage with regard to the unexercised animals (Groups I—VI) as well as the exercised animals of Group VI.

The Number of Cells per Unit Volume of Tissue.

The number of cells of the different zones of the humerus and the scapula are expressed in proportion to the same unit volume of tissue. The unit of volume used is equal to 10 "rectangles" (see Fig. 2, p. 26) of a section 15 μ thick, i.e. $10 \times 125 \times 12.5 \times 15$ cubic microns.

The results on the humerus are given in Table 2 and Fig. 6, and those on the scapula in Table 3 and Fig. 7.

TABLE 2.

The Humerus. The Number of Cells per Unit Volume.

Groups		Tangential Zone			Transitional Zone			Radial Zone		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	73	93.0	—	73	49.3	—	73	22.5	—
Exercised:	II	10	102.0	9.0±6.8	10	60.4	11.1±2.0	10	25.2	2.7±1.1
	III	10	101.2	8.2±7.0	10	55.9	6.6±2.1	10	23.5	1.0±1.1
	IV	10	97.4	4.4±7.0	10	51.0	1.7±2.1	10	23.8	1.3±1.1
	V	10	112.8	19.8±6.8	10	52.7	3.4±2.3	10	23.2	0.7±1.1
	VI	17	89.6	−3.4±5.6	17	38.9	−10.4±1.7	17	18.1	−4.4±0.8

TABLE 3.

The Scapula. The Number of Cells per Unit Volume.

Groups		Tangential Zone			Transitional Zone			Radial Zone		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	73	101.0	—	73	54.8	—	73	17.3	—
Exercised:	II	10	103.2	2.2±9.2	10	64.1	9.3±3.2	10	19.9	2.6±0.9
	III	10	96.4	−4.6±9.2	10	61.6	6.8±3.3	10	19.8	2.5±1.0
	IV	10	112.8	11.8±9.0	10	57.1	2.3±3.2	10	17.3	0.0±0.9
	V	10	101.2	0.2±9.2	10	57.0	2.2±3.2	10	17.2	−0.1±0.9
	VI	17	86.4	−14.6±7.4	17	43.5	−11.3±2.6	17	14.5	−2.8±0.8

In studying the diagrams we find two apparent facts. At first we can see a certain regularity mostly manifested in the transitional and the radial zone of the two cartilages. This consists of an increase of the number of cells per unit volume in Group II and then, in connection with continuous exercise, of a rather succesive decrease until the number of cells in Group VI reveal a statistically significant lower value compared with the control-material. Some indication of an agreement with this picture is also found in the tangential zones.

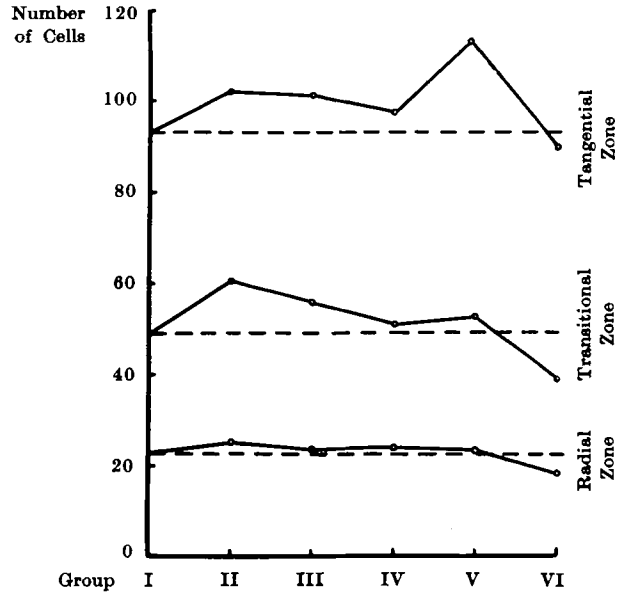


Fig. 6. The humerus. The number of cells per unit volume. ——— = exercised animals. ····· = unexercised animals.

Without doubt the transitional zones primarily attract our attention, and especially the increase of the number of cells in Group II. The numerical value of this increase is higher than those of the other two zones, both as to the humerus as well as to the scapula. It is statistically significant in the humerus, and nearly so in the scapula. In the humerus, Group III, there is also a statistically significant increase of the number of cells.

If the increase of the number of cells of each zone in Group II is expressed in percentage of the value of the corresponding zone of the unexercised animals, it will be seen that now also the transitional zones reveal the highest increase in comparison with the other zones. In the case of the humerus it is 20 per cent, and in the scapula 17 per cent.

In the radial zones, Group II, there are statistically very probable differences between the unexercised and the exercised animals. However, the numerical values of these are low.

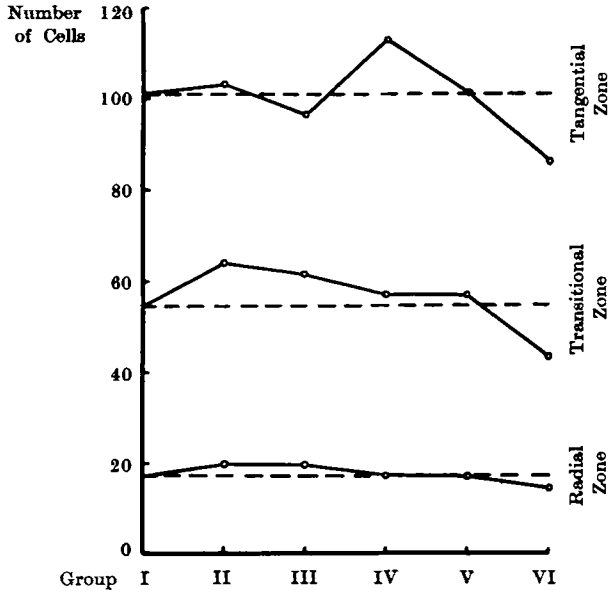


Fig. 7. The scapula. The number of cells per unit volume. ——— = exercised animals. ····· = unexercised animals.

In the tangential zone of the humerus and the scapula the other fact mentioned appears. This is an irregularity between the different groups as to the number of cells per unit volume. Especially surprising are Group V of the humerus and Group IV of the scapula, each revealing relatively great numerical deviations from the control-material without any correspondence in the other zones. As no differences of the tangential zones were statistically significant, the cause of these deviations is to be sought eventually in a chance selection of the animals, which might have affected the results owing to the small number of animals in these groups. Furthermore, it is possible that a more pronounced shrinkage of the superficial part of the cartilage in this groups had occurred. But at least in the case of the humerus in Group V the increase of the average volume (see Fig. 9) seems to tell against this consideration. However, there is also another question which of necessity comes to the fore in this connection: — the sources of error connected with the counting of

TABLE 4.

The number of Chondrons in the Transitional Zone Having 2, 3, . . . etc. Cells.

Groups		2 Cells	3 Cells	4 Cells	≧ 5 Cells	Total
				Humerus		
Unexercised:	I—VI	23.1	8.9	2.9	1.1	36.0
Exercised:	II	20.9	16.6	5.8	2.8	46.1
	III	21.6	11.5	4.1	1.7	38.9
	IV	22.2	9.9	2.7	2.1	36.9
	V	23.2	14.4	5.8	2.7	46.1
	VI	20.6	8.9	3.0	0.9	33.4
				Scapula		
Unexercised:	I—VI	22.7	4.1	0.7	0.1	27.6
Exercised:	II	26.0	8.2	2.8	0.6	37.6
	III	22.0	6.3	1.1	0.3	29.7
	IV	22.1	5.0	0.7	—	27.8
	V	27.2	6.7	1.3	0.4	35.6
	VI	18.1	3.6	1.1	0.1	22.9

TABLE 5.

Differences Between Each Exercised Group and the Control-Animals as to the Number of Chondrons Having More than 2 Cells as a Percentage of the Total Number of Chondrons Registered in the Transitional Zone.

A positive difference means a higher value for the exercised group.

Groups				
II	III	IV	V	VI
		Humerus		
19.8±4.2	8.6±4.3	4.8±4.2	12.3±4.4	3.3±3.3
		Scapula		
11.6±2.9	5.5±3.1	−0.9±3.0	4.3±3.0	1.0±2.5

the cells in this very zone, mentioned earlier. So it does not seem to be out of the question that such sources of error may more or less have influenced these results. The problem as to the number of cells per unit volume in the tangential zones will be discussed in greater detail further on in the present work.

Number of Cells per Chondron.

The number of cells per chondron was registered in all the zones of the humerus and the scapula.

In the statistical treatment of the number of cells per chondron in the tangential and radial zones, respectively, even probable differences did not appear between the various exercised groups and the control-material, neither in the humerus nor the scapula.

An analysis of the transitional zone, on the contrary, gave other results. From Table 4 and Fig. 8 it appears that, in the humerus, the number of chondrons having 3, 4, and $\geqq 5$ cells, respectively, shows an increase in Groups II and V of the exercised animals. In

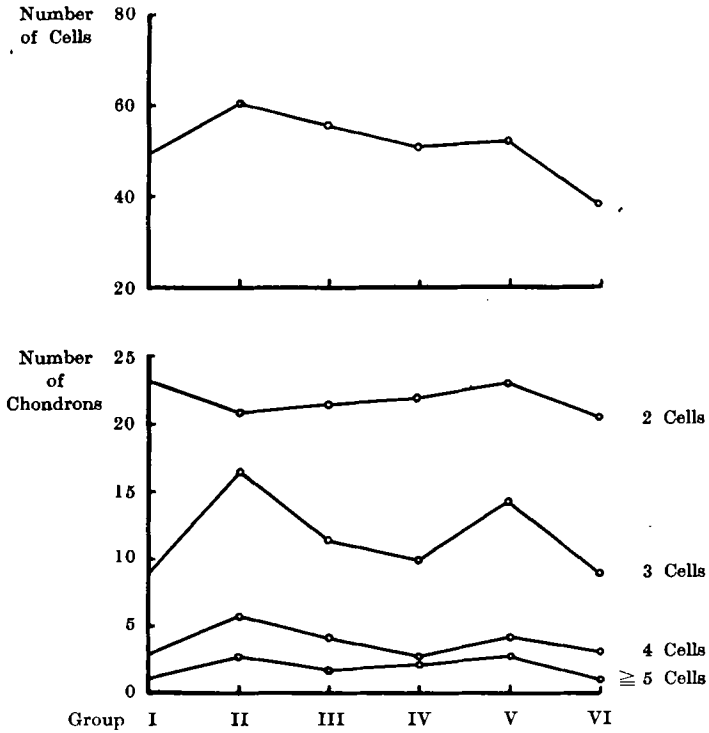


Fig. 8. The upper diagram shows the number of cells per unit volume in the transitional zone of the humerus of the exercised animals. The lower diagram gives the number of chondrons having 2, 3, ... etc. cells registered in the corresponding zone.

the scapula we find the same condition in the corresponding groups, but besides this a slight increase in the number of chondrons having 2 cells.

If the number of chondrons having more than 2 cells is expressed in percentage of the total number of chondrons registered, and then a calculation is made of the difference between each exercised group and the unexercised control-material, the results, given in Table 5, will be obtained.

In judging the figures now given, the sources of error connected with the registering method used must be kept in mind (see p. 41—42). However, the relative parallelism between the different curves in Fig. 8 — also to be found in the scapula (see Table 4) — speaks in favour of the fact that an increase of the number of chondrons having many cells actually exists in Group II, and eventually also in Group V.

Such an increase of the number of chondrons having many cells in certain groups was not to be observed in the unexercised animals. The same fact applies to the parallelism described.

Furthermore, Fig. 8 shows the relation between the number of cells per unit volume and the number of different chondrons in the transitional zone. The number of cells and the number of chondrons do not refer to the same unit volume of tissue, wherefore no numerical comparisons can be drawn between them. Nevertheless, the diagrams give valuable information. Briefly expressed, there is an obvious parallelism also between the number of cells per unit volume and the number of cells per chondron in the various groups. The condition is the very same regarding the scapula.

Average Volume of Cells.

Each method, hitherto known, of determining the volume of individual cells involves a schematization. This also pertains to the present method, as mentioned earlier. Accordingly, the figures obtained are not equal to the real volumes of the cells. Nevertheless they seem to allow comparisons to be drawn between unexercised and exercised animals.

The results of these comparisons are to be seen in Tables 6 and 7,

TABLE 6.

The Humerus. The Average Volume of Cells (in μ^3).

Groups		Tangential Zone			Transitional Zone			Radial Zone		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	73	251.7	—	73	413.6	—	73	354.2	—
Exercised:	II	10	256.3	4.6 ± 22.5	10	417.6	4.0 ± 35.1	10	381.3	27.1 ± 28.8
	III	10	243.6	-8.1 ± 22.5	10	449.3	35.7 ± 36.3	10	305.9	-48.3 ± 28.8
	IV	10	244.2	-7.5 ± 21.3	10	441.8	28.2 ± 36.3	10	403.2	49.0 ± 30.0
	V	10	292.6	40.9 ± 22.5	10	500.0	86.4 ± 36.9	10	419.9	65.7 ± 28.8
	VI	17	309.3	57.6 ± 17.9	17	565.6	152.0 ± 30.5	17	458.5	104.3 ± 24.2

TABLE 7.

The Scapula. The Average Volume of Cells (in μ^3).

Groups		Tangential Zone			Transitional Zone			Radial Zone		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	73	219.5	—	73	269.0	—	73	262.1	—
Exercised:	II	10	220.6	1.1 ± 21.9	10	278.2	9.2 ± 24.2	10	248.3	-13.8 ± 20.7
	III	10	206.2	-13.3 ± 22.5	10	248.3	-20.7 ± 24.8	10	250.0	-12.1 ± 20.7
	IV	10	210.2	-9.3 ± 21.9	10	281.1	12.1 ± 24.2	10	246.0	-16.1 ± 20.7
	V	10	218.9	-0.6 ± 22.5	10	298.4	29.4 ± 25.3	10	284.5	22.4 ± 21.3
	VI	17	248.8	29.3 ± 18.4	17	312.2	43.2 ± 20.2	17	294.3	32.2 ± 16.1

and Figs. 9 and 10. The average volume of the cells in the different zones of the humerus shows an increase in relation to the length of the exercise. As seen from Table 6 the differences between exercised and unexercised animals are not statistically significant, except for all the three zones in Group VI.

On the contrary, the average cell-volume of each zone of the scapular cartilage does not reveal any statistically significant increase in the exercised animals.

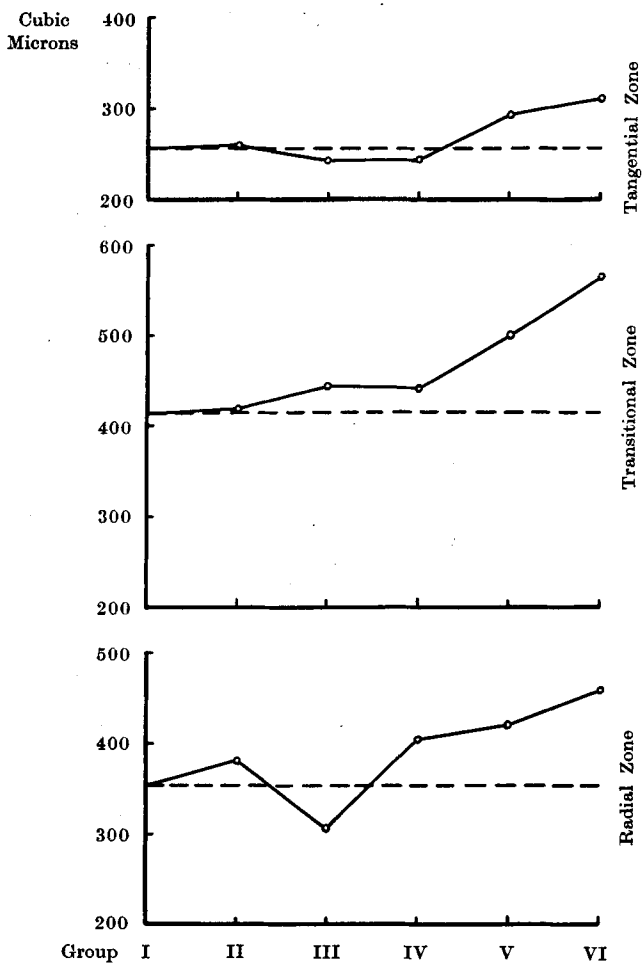


Fig. 9. The humerus. The average volume of cells.
——— = exercised animals. ····· = unexercised animals.

The decrease of the value of the average volume found in Group III in the radial zone appears not to be statistically significant.

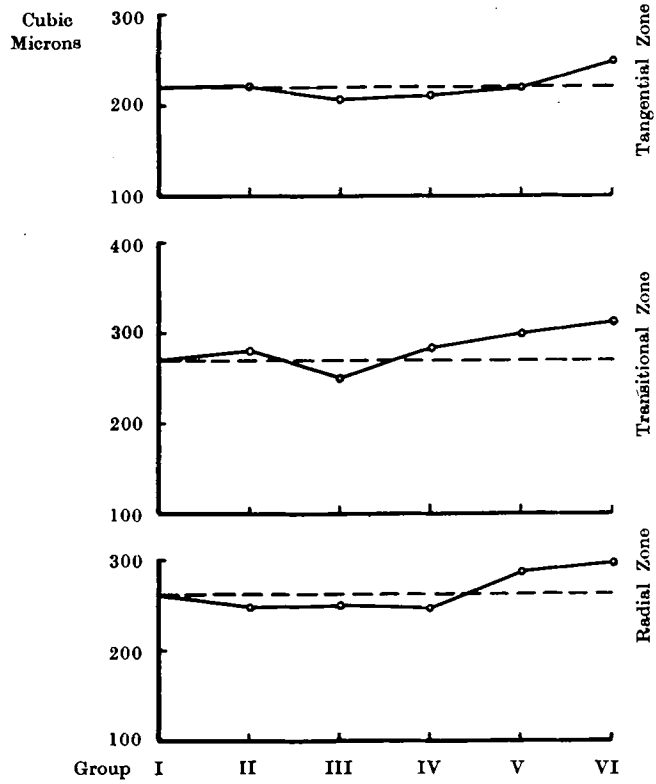


Fig. 10. The scapula. The average volume of cells.
——— = exercised animals. ····· = unexercised animals.

The Volume of Cellular Substance per Unit Volume of Tissue.

As the number of cells per unit volume as well as the average volume of the cells revealed changes in connection with exercise, it was of interest to examine how the volume of the cellular substance per unit volume of tissue was influenced by these variations. The unit volume of tissue used here is, of course, the same as mentioned on p. 50.

The volume of the cellular substance was calculated with regard to each zone of every individual articular cartilage. In so doing, the

TABLE 8.

The Humerus. The Volume of Cellular Substance (in μ^3) per Unit Volume of Tissue.

Groups		Tangential Zone			Transitional Zone			Radial Zone		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	73	23443	—	73	19987	—	73	7914	—
Exercised:	II	10	26093	2650±2788	10	25114	5127±1440	10	9539	1625±668
	III	10	24503	1060±2811	10	24958	4971±1521	10	7160	−754±674
	IV	10	23881	438±2742	10	21917	1930±1423	10	9360	1446±662
	V	10	32959	9516±2892	10	25822	5835±1538	10	9717	1803±697
	VI	17	26922	3479±2154	17	21594	1607±1175	17	8410	496±582

TABLE 9.

The Scapula. The Volume of Cellular Substance (in μ^3) per Unit Volume of Tissue.

Groups		Tangential Zone			Transitional Zone			Radial Zone		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	73	21738	—	73	14354	—	73	4510	—
Exercised:	II	10	21266	−472±2454	10	17718	3364±1117	10	4925	415±386
	III	10	19642	−2096±2580	10	15183	829±1169	10	4913	403±386
	IV	10	23604	1866±2500	10	16007	1653±1135	10	4176	−334±380
	V	10	21404	−334±2529	10	16779	2425±1146	10	4861	351±392
	VI	17	21416	−322±2212	17	13357	−997± 904	17	4274	−236±311

number of cells per unit volume was multiplied by the average volume of the cells in the same zone of the cartilage. The individual values obtained in this way were then used as a basis for the statistical treatment of each group.

The volume of the cellular substance per unit volume of tissue is given in cubic microns in Tables 8 and 9. In Table 10 and Figs. 11 and 12 the same volume is converted into the percentage of cellular substance of the tissue.

TABLE 10.

The Percentage of the Cellular Substance of the Cartilage.

	Unexercised I—VI	Exercised				
		II	III	IV	V	VI
			Humerus			
Tangential Zone	10.0	11.1	10.5	10.2	14.1	11.5
Transitional Zone	8.5	10.7	10.6	9.4	11.0	9.2
Radial Zone	3.4	4.1	3.1	4.0	4.1	3.6
			Scapula			
Tangential Zone	9.3	9.1	8.4	10.1	9.1	9.1
Transitional Zone	6.1	7.6	6.5	6.8	7.2	5.7
Radial Zone	1.9	2.1	2.1	1.8	2.1	1.8

As seen from these tables and figures, the cellular substance shows a significant increase in the transitional zone of the humeral cartilage in the exercised Groups II, III, and V as well as in Group V in the tangential zone as compared with the control-material.

The scapular cartilage reveals a statistically significant increase of the cellular substance only in Group II of the transitional zone.

It is also of interest to draw a comparison between the unexercised animals and Group VI of those exercised (Table 10). It then appears that the ratio between the cellular and the intercellular substance in Group VI again, on the whole, turned out to be the same as that of the unexercised animals.

Finally, as to the tangential zone of the humeral cartilage, Group V, some words may be mentioned about its volume of cellular substance. Here there is a statistically significant difference in relation to the unexercised animals, probably depending on the fact that two differences that were not statistically significant in themselves (as to the number and average volume of cells) had been combined.

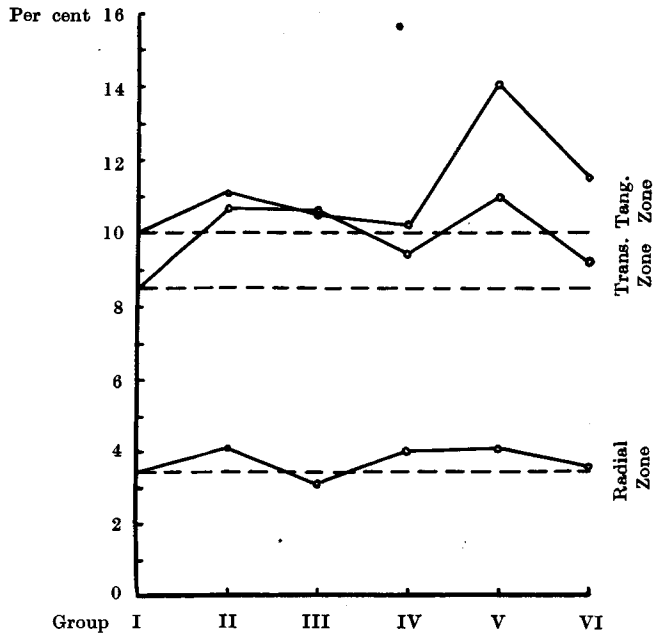


Fig. 11. The humerus. The percentage of the cellular substance of the cartilage. ——— = exercised animals. ····· = unexercised animals.

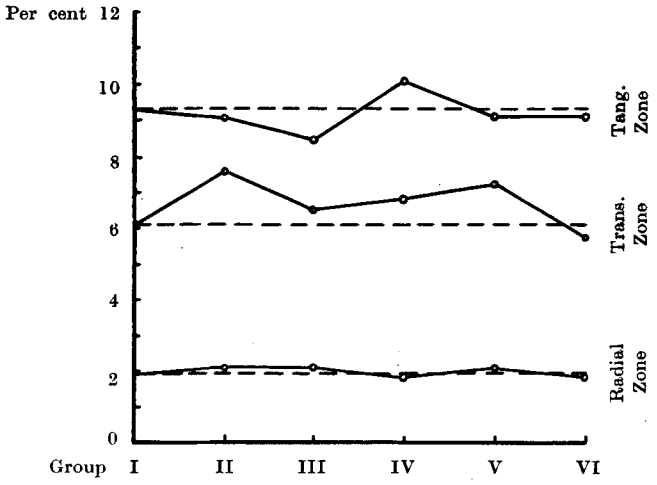


Fig. 12. The scapula. The percentage of the cellular substance of the cartilage. ——— = exercised animals. ····· = unexercised animals.

TABLE 11.

The Humerus. The Thickness of the Uncalcified Cartilage (in μ).

Groups		Cartilages with Visible Limits			Cart. with and those without Visible Limits		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	56	190.3	—	73	192.7	—
Exercised:	II	8	193.4	3.1 ± 8.6	10	194.2	1.5 ± 7.8
	III	6	209.8	19.5 ± 9.4	10	205.9	13.2 ± 7.8
	IV	7	181.7	− 8.6 ± 8.6	10	194.2	1.5 ± 7.8
	V	7	177.8	−12.5 ± 8.6	10	185.6	−7.1 ± 7.8
	VI	12	186.4	− 3.9 ± 7.0	17	199.7	7.0 ± 7.0

TABLE 12.

The Scapula. The Thickness of the Uncalcified Cartilage (in μ).

Groups		Cartilages with Visible Limits			Cart. with and those without Visible Limits		
		No.	Mean	Difference between unexercised and exercised animals	No.	Mean	Difference between unexercised and exercised animals
Unexercised:	I—VI	58	253.5	—	73	256.6	—
Exercised:	II	7	248.0	− 5.5 ± 17.2	10	248.8	− 7.8 ± 14.0
	III	8	269.9	16.4 ± 14.8	10	259.7	3.1 ± 13.3
	IV	8	235.6	−17.9 ± 14.8	10	245.7	−10.9 ± 13.3
	V	8	247.3	− 6.2 ± 14.8	10	247.3	− 9.3 ± 13.3
	VI	12	275.3	21.8 ± 14.0	17	288.6	32.0 ± 11.7

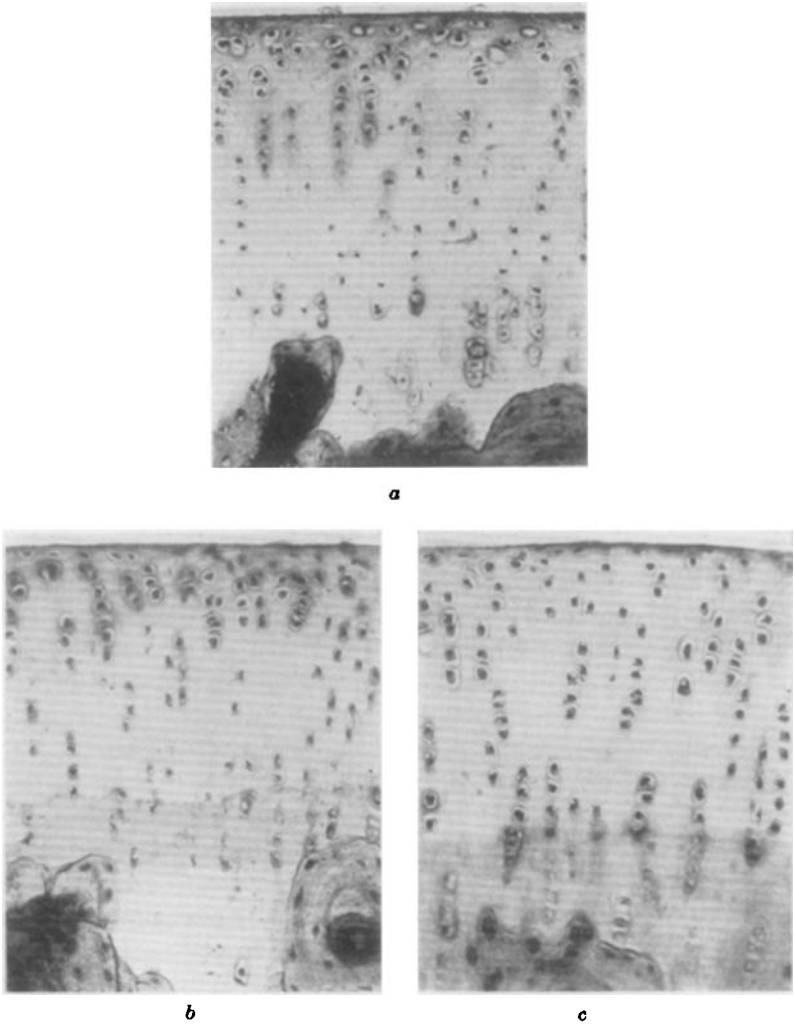
The Thickness of the Uncalcified Cartilage.

As mentioned earlier about 25 per cent of the articular cartilages examined did not show any visible limit between the calcified and the uncalcified cartilage at the point where the measurement was to be carried out. Nevertheless, the position of a "limit" could be determined by means of a special procedure (p. 32), and the error of this method was relatively small. As it was possible that the part

of the material showing a visible limit could have represented a selection with respect to the characteristic examined, i.e. the thickness of the cartilage, the statistical calculations were carried out partly on cartilages having visible limits, and partly on the whole material.

As seen from Tables 11 and 12, there are no statistically significant differences between the various exercised groups and the control-material. With the exception of Group VI of the scapula, the results are the same whether they are obtained only from cartilages having visible limits, or from those of the whole material. In this group, however, there is a very probable difference between exercised and unexercised animals when the thickness of cartilage is calculated from the whole material. However, there is some doubt as to whether this difference is really to be regarded as representing an increase of the thickness of the scapular cartilage in this exercised group. As a matter of fact, in four of the five cartilages where it was difficult to determine the limit, a thickness was registered exceeding the mean of the group. Moreover, there was no increase of the thickness of the cartilage in the previous groups — a fact also contributing to our doubt as to whether an increase of the thickness of the cartilage had really taken place in Group VI.

Another fact of interest in this connection was observed in the microscopical examination of the sections. It was revealed that a well-marked limit between the uncalcified and the calcified cartilage could always be seen (except in one case) within the non-weight-bearing part of the articular cartilage of the humerus. This also pertained to the 25 per cent of the cartilages where no such limit could be distinguished within the weight-bearing part. The cause of this may possibly be found in various chemical compositions of the intercellular substance, eventually due to the different functional conditions in which these parts of the articular cartilage have existed during life.



a

b

c

Fig. 13. Humeral cartilages of three different groups, indicating some of the results mentioned above.

a. Group I, unexercised. *b*. Group II, exercised for 20 days. In the transitional zone, a short distance below the articular surface, there are more cells than in '*a*'. *c*. Group VI, exercised for 100 days. Here there are fewer cells in the transitional zone than in '*a*' and '*b*'. Furthermore the cells are larger in this cartilage.

All the photos are taken within the weight-bearing part of the cartilage, at + 60°. Haematoxylin and eosin stain; × 155.

General Discussion

The present investigation was carried out on male guinea-pigs which had lived normally in their cages up to an age of 11 $^1/_2$ months. After that, a number of them were subjected to systematical exercise. With the exception of this difference the whole material was uniformly treated, as far as possible, with regard to the histological technique and other methods. On account of this, it may be hoped that the differences observed in the articular cartilages of exercised and unexercised animals are also an expression of differences in the cartilage during life. The sources of error in these methods have been discussed earlier.

In the previous chapter there is a description of a number of such differences in the structure of the articular cartilage between exercised and unexercised animals. Furthermore, it appeared that these changes varied according to the length of the period of exercise. In the present chapter an attempt will be made to interpret the observations described.

The number of cells per unit volume and per chondron. The animals exercised for about three weeks (Group II) revealed an increase of the number of cells per unit volume. This was most pronounced in the transitional zone, both numerically and as a percentage; and it was statistically significant as to the humerus, and nearly so in the scapula too, the difference in the latter being equal to 2.9 times its standard error. Moreover, it was established that there was an increase of the number of cells per chondron in the same zone and group. These two observations have been interpreted in such a way that there was a reproduction of the cells within the chondrons of the transitional zone.

This conclusion is supported by some data in earlier literature. Thus Ogston (1876) spoke of "the focus of central growth," and

HAMMAR (1892) as well as ELLIOT (1936) observed changes of the shapes of the nuclei of cells, which were most frequent within a region mainly corresponding to the transitional zone. They interpreted these nuclei as signs of eventual amitotic cell-divisions, though no real cell-division was ever seen. ELLIOT found a greater number of such nuclei in the articular cartilage of exercised dogs than in that of rabbits kept in a state of rest. Such a comparison between dogs and rabbits, however, seems to be quite inconclusive.

Figs. 6 and 7 showed that, in connection with a continuation of the exercise, the number of cells per unit volume decreased in the transitional and the radial zone. Also this decline was most pronounced in the transitional zone where it ran parallel with a decrease of the number of cells per chondron (Table 4 and Fig. 8). The most plausible explanation of this phenomenon seems to be an (increased) production of intercellular substance. In the microscopical examination of the histological sections it is also often seen how a cell or two, which have in all appearance belonged to an adjacent chondron, are separated from the latter by a thin lamella of intercellular substance. These lamellae seem to increase in thickness, resulting in the cells being more and more removed from each other. In consequence of the production of this intercellular substance, particularly between the cells within the chondrons, the latter are divided into smaller units. This process is reflected in that decrease of the number of cells per chondron which was observed in the registration. On the other hand, this dispersion of the cells must lead to a decrease of their number per unit volume unless a continuous reproduction of the cells keeps pace with the formation of the intercellular substance.

The process described also seems to agree with the current opinion that the cells take part in the production of intercellular substance. The present investigation, however, gives no information as to the mechanism of this production.

The successive decrease of the number of cells per unit volume in the transitional zone seems to be impeded a little in Group V. This fact is perhaps not of such great importance. Possibly it only depends on a chance selection of the animals of this group. However, a study of Fig. 8 and Tables 4 and 5 reveals that, in the transitional zone in Group V itself, there is a certain increase of the number of

cells per chondron with regard to the humerus as well as to the scapula. Hence, with a reference to the observations in Group II, it does not seem to be entirely excluded that the facts found in Group V indicate a slightly more pronounced reproduction of cells. This would eventually have been statistically significant in a larger material. However, this problem must now be left unanswered.

Accordingly, in Group II (and eventually in Group V), a reproduction of cells has been ascertained in the transitional zones in connection with the exercise. Now the question comes to the fore as to whether the increase of the number of cells per unit volume indicated in some groups, with regard to the tangential and the radial zones, also depends on a reproduction of the cells within these zones. The present investigation gives no definitive answer to this question. Nevertheless certain data present us with some information.

In the case of the tangential zone the most actual groups are Group II and Group V of the humerus as well as Group IV of the scapula. No differences between the control-material and these groups were statistically significant as to the number of cells per unit volume. In addition to this, no increase of the number of cells per chondron could be shown in the exercised animals. Of course, this does not exclude the possibility of a reproduction of cells in this zone; but, on the other hand, it decidedly seems to speak in favour of such a reproduction being necessarily limited. This view is furthermore supported by the fact that HAMMAR (1892) and ELLIOT (1936) found practically no signs of amitotic cell-division in the most superficial parts of the cartilage.

What other possibilities can then be thought of as being the cause of an increase of the number of cells in the tangential zone? Earlier methodological difficulties and chance selection have been mentioned as presumable causes of variation of the number of cells in this zone. A third factor, however, seems to be of greater importance in this respect. There may be no doubt but that the process occurring in the transitional zone results in a successive displacement of cells from this zone up into the tangential one. Such a displacement has earlier been assumed by OGSTON (1876) and HAMMAR (1892), amongst others.

If there had been several exercised groups prior to Group II, it is

possible that we should have been able to prove that the increase of the number of cells would have appeared first in the transitional zone, and later in the tangential one. This would have given further corroboration to the view of a displacement of the cells from the transitional to the tangential zone.

In the radial zones we found a slight increase of the number of cells per unit volume in Group II (Tables 2 and 3, and Figs. 6 and 7). The numerical values of these differences were low, but statistically very probable both as to the humeral and the scapular cartilage. Nor has it been possible in these zones to show any increase of the number of cells per chondron. The reason for this may perhaps be that this zone has so few cells. On the other hand, there is a very striking similarity of the course of the curves that represent the transitional and the radial zones respectively. As no systematic error in this method is known which could explain this similarity, and as it seems to be very unlikely that a displacement of the cells from the transitional zone into the middle of the radial one should occur, and, finally, as ELLIOT (1936) found signs of amitotic cell-division, though scanty, in the interior of the cartilage, the only plausible explanation of this increase may be a reproduction of cells within the radial zone itself.

The average volume of the cells. The average volume of the cells of the humeral cartilage revealed an increase in all the three zones in connection with the exercise. A similar condition was not observed in the scapular cartilage. A satisfactory explanation as to the divergency of the results from the humerus and the scapula is difficult to find.

In the main, it is not possible to make a complete analysis of the effect of the exercise on the size of the cells solely from the results acquired in this investigation. In fact, it is quite impossible that the successive increase of the average volume of cells found in the humeral cartilage of exercised animals should be able to continue beyond a certain limit. For that reason, a longer period of observation would have been greatly desirable in order to obtain a definite conception of the connection between the functional strain on the cartilage and the sizes of its cells.

In literature there is no information whatever as to these problems regarding adult articular cartilage. In the investigation on growing rabbits carried out by HOLMDAHL and INGELMARK (1948), there were certain indications that the sizes of the cells had increased in the exercised animals.

The proportion between the cellular and the intercellular substance. In consequence of the variations of the number of cells per unit volume and the sizes of the cells described above, the proportion between the cellular and the intercellular substance also showed some changes. Except for the tangential zone of the humeral cartilage in the case of Group V, already discussed, this proportion was kept relatively constant in the tangential and the radial zone of the humerus and the scapula, respectively. In the transitional zone, however, there were statistically significant differences between the exercised and the unexercised animals, both as to the humeral and the scapular cartilage. Thus it appeared that there was a shift in this proportion in favour of the cellular substance as early as in Group II. In principle, this condition existed up to Group VI, where the cellular substance was again reduced in proportion to the intercellular.

With reference to Figs. 6 and 7 as well as to 9 and 10, the increase of the cellular substance in the beginning of the exercise-period depends on a multiplication of the cells. Though the number of cells per unit volume subsequently diminishes, this does not bring about a corresponding decrease of the volume of the cellular substance per unit volume of tissue since the continued exercise, especially concerning the humerus, simultaneously causes an increase of the average volume of the cells.

The results in Group VI seem to be of special interest. None of the various zones of the humerus and the scapula reveals any statistically significant difference between exercised and unexercised animals.

The results of the present investigation do not agree with those obtained in connection with the exercise of growing animals. Thus HOLMDAHL and INGELMARK (1948) found that the proportion between the cellular and the intercellular substance was changed in favour of the latter in exercised animals. The cause of this discrepancy of the results will be discussed later.

The thickness of the uncalcified cartilage. In spite of the variations of the structure of the uncalcified cartilage observed during the course of the exercise, the thickness of this cartilage remained relatively unchanged. So the comparison between exercised and unexercised animals as to the humeral cartilage did not reveal any differences from a statistical point of view, while eventually an increase of the thickness of the scapular cartilage had occurred in Group VI. As to this increase, however, see the previous chapter, p. 64.

In judging these results it would have been of great value to have had access to comparable observations in earlier literature. Such are, however, lacking. Earlier workers have studied the thickness of the articular cartilage in various joints, and in different parts of the same cartilage, and discussed some factors that may be thought of as influencing this thickness (WERNER, 1897; FICK, 1904; BENNINGHOFF, 1922), amongst others. A theoretical-geometric analysis of the thicknesses of articular cartilages has been carried out by BACKMAN, EDLÉN, HJORTSJÖ, and LÖFGREN (1948). These works, however, have no immediate connection with the actual results of the present investigation.

On the other hand, it is of interest to compare our observations with the findings of RETTERER (1908) as well as those of HOLMDAHL and INGELMARK (1948). The former observed a thicker articular cartilage in guinea-pigs in connection with a greater functional strain on the joints during their period of growth. The latter workers proved that a statistically significant increase of the thickness of the articular cartilage really arose in connection with the exercise of growing rabbits. Accordingly, these observations seem to contradict those of the present investigation. How these discrepancies are eventually to be explained, will be dealt with further on in this discussion.

To sum up, the following may be said:

1. In the discussion on the number of cells per unit volume and the number of cells per chondron in the different groups, as a whole, it was found that, concerning the transitional zones, the increase of the number of cells in the beginning of the exercise (Group II)

must be caused by a reproduction of cells in this zone. In the radial zone it is very likely that there was a reproduction of cells, though relatively slight. If the latter did occur in the tangential zone, however, it appeared to be very limited. The cells in this zone mainly seem to originate from those of the transitional zone through a secondary displacement.

2. The decrease of the number of cells per unit volume and per chondron, concomitant to the continued exercise, appears to depend on a more marked formation of intercellular substance, whereby the cells are separated from each other.
3. In the humeral cartilage the sizes of the cells grew larger during the course of the exercise. In the scapular cartilage no corresponding increase could be observed.
4. The proportion between the cellular and the intercellular substance was temporarily changed in the transitional zone in favour of the cellular one. In the last group exercised, however, there was on the whole the same proportion as in the unexercised animals with respect to all the zones examined.
5. The thickness of the uncalcified cartilage of the humerus was practically constant in the various exercised groups, and was the same as in the control-animals. The scapular cartilage showed a slight, statistically very probable, increase in the last group exercised. Yet, some reservations were made for this value.
6. Accordingly, the changes observed in the different zones did not result in any definite increase of the thickness of the cartilage, but implied a structural modification of the articular cartilage.

In order to make a more thorough analysis of the implication of the changes occurring in the different zones in relation to the cartilage as a whole, it would have been necessary to have a rather exact knowledge of the thicknesses of these zones in the various groups as well as of the amount of the products of wear and tear of the articular surface. It has not been possible, however, to carry out such determinations.

Some of the factors which may have influenced the results. The effects of the histotechnical treatment which might have affected the

results have been mentioned earlier, and will not be dealt with any more.

Of other factors to be taken into account as influencing the results of an investigation of this kind, the arrangement of the exercise is naturally of great importance. In the review of literature it was mentioned that extremely pronounced changes in the functioning of a joint could cause damages in the cartilage, which is also stressed by FREUND (1939). HOLMDAHL (1942) has formulated the relation of the function to the tissues of a joint in such a way that a hypofunction, or an afunction, as well as a hyperfunction is detrimental to the tissue, whereas a normal function maintains and strengthens it.

But also an increase of function kept within physiological bounds can be varied in different ways, e.g. with regard to intensity and duration. Especially the relatively sudden increase of function seems to have been of importance to the present results. It is plausible that the obvious increase of the number of cells in the transitional zones would not have appeared if the animals had been adapted more slowly to their exercise. Moreover, it was mentioned that a shortening of the intervals between the exercised groups in the beginning of the experiment might have given us additional information about the reactions in the various zones. But it has also been indicated that, if the exercise had been continued longer than that of Group VI, the final results might have been different.

The proportion between the cellular and the intercellular substance, as well as the number of cells per chondron in Group VI, has on the whole reverted to the values represented by the unexercised animals. However, neither the values of the number of cells per unit volume nor those of the average volume of the cells seemed to have been stabilized. The facts mentioned, however, lead our thoughts to the possibility that the cartilage was passing over into a stable stage of adaptation to the very increase of the function applied.

Whether the changes observed in the cartilage were results of an immediately stimulating effect of the function on this tissue, is difficult to determine. Even ROUX (1895) and FICK (1904) were of the opinion that the "kneading" of the articular cartilage in the functioning of a joint has an immediate, stimulating effect on the formation

of the cartilage. This view is later often met with in subsequent literature.

However, some investigations, mainly on growing but also on adult animals, by M. SILBERBERG (1936), and M. SILBERBERG and R. SILBERBERG (1938—41) have shown that the structure of the articular cartilage was affected if the animals were treated with various hormones. Though the results obtained after such treatments can hardly be designated as physiological, yet they prove that other factors than those of a merely mechanical character may be of some importance. The real mechanism behind the changes in the articular cartilage of the present investigation is not known. But it may be justifiable to claim that they have arisen in immediate connection with the increased functioning of the joints that was applied.

As mentioned above, the present results differ from the observations made by earlier research workers concerning the proportion between the cellular and the intercellular substance as well as concerning the thickness of the cartilage in exercised animals. However, it should be stressed that it is only the results of Group VI and those of the control-animals of the present material that can be compared with the results from earlier investigations, the latter having no correspondence to the exercised Groups II—V of the present material.

What may then be the causes of these discrepancies? Eventually they may be sought for in the different methods employed; but this possibility seems to be hardly plausible, since the various methods appear to be fully practicable when it was only a question of drawing comparisons. A divergency in the treatment of the material, however, should be adduced. Earlier the various zones of the uncalcified cartilage have not been examined separately as in this investigation.

On the other hand, another factor seems to be of the greatest importance in this connection. Earlier investigations were carried out on growing animals, whereas the present work deals only with adult animals.

In a growing animal the tissues are in a stage of development. The effect of an increased function on such a tissue may therefore be assumed to depend on the capacity of the tissue to react on mechanical stimuli, but also on the factors that condition a normal growth. More-

over, it may be thought that an exercise also eventually influences the latter factors.

In an adult articular cartilage, on the contrary, its proper growth is completed. This cartilage is in a more stable condition, where a certain balance is assumed to exist between its wear and tear and a continuous regeneration. For this reason, the changes observed in such a tissue in connection with an increased function might be an expression of the capacity of the tissue to react to external conditions.

Consequently, the factor of age may be the most important one when it comes to explaining the discrepancies of the results of the various investigations. Against this background there is instead a possibility that the divergent results will throw light upon the way the articular cartilage reacts during different periods of the life of an individual.

Applications of the results. The results obtained in this investigation are primarily applicable to the shoulder-joint of adult guinea-pigs under the experimental conditions indicated. However, it seems probable that they can also contribute to an understanding of the reactions within the weight-bearing part of other adult articular cartilages.

At first this implies that an adult articular cartilage does react on an increased function of a joint. Both the cellular and the intercellular component are involved, the former appearing to react first by a multiplication of the cells mainly in the transitional zone, whereas the intercellular substance seems to be somewhat later.

But the fact that the various zones of the cartilage proved to have varying degrees of reaction and, furthermore, that the reaction varied in accordance with the length of the exercise, has given a turn to the results whereby it is possible to make a few additional applications of a general character.

In the review of literature we found that the functioning of a joint was accompanied by a wear and tear of the surface of the articular cartilage, increasing with a more intensive and longer functional strain. It must be assumed that such a process of wear and tear was present in the joints examined, too. But it has also been considered

self-evident that such a wear of the cartilage must be restituted by fresh cartilaginous substance. And now it seems as if the results of the present investigation can elucidate this process of *continuous regeneration.*

With reference to what has earlier been adduced in this discussion it is unmistakable that the transitional zone plays a very great rôle in this process. In this investigation it was possible to prove that incomparably the greatest part of the reproduction of cells in the cartilage takes place in this zone. Through the concomitant formation of intercellular substance the cells are separated from each other, which appears to result in a secondary displacement of produced cartilaginous substance towards the articular surface, whereby the products of wear and tear are compensated.

In the radial zone a slight reproduction of cells seems to occur with a concomitant formation of intercellular substance. This process, however, may not be of such a magnitude as to contribute to compensating the loss of substance on the articular surface.

In the actual investigation there was no definite increase in thickness of the uncalcified cartilage in exercised animals. This implies that the wear and tear and the continuous regeneration kept pace with each other. It would, however, be too rash to conclude that this is always the case. Factors such as the intensity and duration of the exercise as well as the age of the animals might influence the relation between these two processes. Accordingly, if these factors are varied, the result might be an increase of the thickness of the articular cartilage in one case, and a decrease in the other.

The cellular activity ascertained in the transitional zone has also occasioned another reflection. In the earlier experiments made concerning *the repair of artificial defects in the articular cartilage* it seems that only the consideration as to whether the defect penetrated to the marrow cavity or not has been examined. On the contrary, no attention has been paid to the extension in which the various zones of the uncalcified cartilage have been involved. From the illustrations available in works of this kind it appears that, as a rule, the transitional zone has been penetrated completely. It does not seem to be implausible that the minute tendency towards regeneration in these defects is to be associated with this fact, since the continuity of the

transitional zone is broken and the tendency towards a multiplication of the cells in the radial zone is slight.

If the defects of the cartilage were very small, FASOLI observed that they had been completely repaired by cartilaginous tissue. Unfortunately it is not recorded in his work how deeply those defects have penetrated. His results, however, may possibly support the above view. The consequence of the latter must be that it is urgent that the transitional zone should be left intact, as far as possible, if an appreciable regeneration of the articular cartilage is to be expected.

TABLE 13.

Mean and Standard Deviation of the Number of Cells per Unit Volume, the Average Volume of Cells, and the Volume of Cellular Substance per Unit Volume of Tissue in the Articular Cartilage of 73 Unexercised Animals: Groups I—VI.

The average volume of cells and the volume of cellular substance are given in cubic microns.

	Tangential Zone		Transitional Zone		Radial Zone	
	$M \pm E(M)$	σ	$M \pm E(M)$	σ	$M \pm E(M)$	σ
	Number of Cells					
Humerus	93.0±2.4	20.2	49.3±0.7	6.0	22.5±0.4	3.1
Scapula	101.0±3.2	27.2	54.8±1.2	9.6	17.3±0.3	2.7
	Average Volume of Cells					
Humerus	251.7±7.5	64.5	413.6±12.7	106.0	354.2±9.8	84.7
Scapula	219.5±7.5	65.7	269.0± 8.6	73.2	262.1±7.5	62.8
	Volume of Cellular Substance					
Humerus	23443±956	8179	19987±495	4239	7914±230	1958
Scapula	21738±876	7453	14354±380	3266	4510±138	1158

TABLE 14.

Mean and Standard Deviation of the Number of Cells per Unit Volume, the Average Volume of Cells, and the Volume of Cellular Substance per Unit Volume of Tissue in the Articular Cartilage of 17 Exercised Animals: Group VI.

The average volume of cells and the volume of cellular substance are given in cubic microns.

	Tangential Zone		Transitional Zone		Radial Zone	
	$M \pm E(M)$	σ	$M \pm E(M)$	σ	$M \pm E(M)$	σ
	Number of Cells					
Humerus	89.6±5.4	22.6	38.9±1.6	6.5	18.1±0.7	2.9
Scapula	86.4±7.0	29.2	43.5±1.9	7.9	14.5±0.7	3.1
	Average Volume of Cells					
Humerus	309.3±18.4	74.9	565.6±34.0	138.8	458.5±27.1	111.7
Scapula	248.8±19.0	78.3	312.2±20.2	82.4	294.3±10.4	42.0
	Volume of Cellular Substance					
Humerus	26922±1693	6970	21594±1169	4821	8410±697	2868
Scapula	21416±2638	10840	13357± 881	3629	4274±276	1140

TABLE 15.

Mean and Standard Deviation of the Thickness (in μ) of the Uncalcified Cartilage of Unexercised Animals: Groups I—VI as well as the Exercised Animals: Group VI.

	Cartilages with Visible Limits			Cart. with and those without Visible Limits		
	No.	$M \pm E(M)$	σ	No.	$M \pm E(M)$	σ
	Unexercised: Group I—VI					
Humerus	56	190.3±3.1	21.8	73	192.7±3.1	23.4
Scapula	58	253.5±5.5	40.6	73	256.6±4.7	39.8
	Exercised: Group VI					
Humerus	12	186.4± 8.6	28.9	17	199.7± 8.6	34.3
Scapula	12	275.3±18.7	64.7	17	288.6±14.0	59.3

TABLE 16.

Differences Between the Various Zones of the Articular Cartilages of the Humerus and the Scapula as well as Between Their Corresponding Zones with Regard to the Number of Cells per Unit Volume, the Average Volume of the Cells, and the Volume of Cellular Substance per Unit Volume of Tissue.

The differences are calculated from Tables 13 and 14. A positive difference implies that the zone first indicated has the highest value.

Difference between	Number of Cells		Average Volume of Cells		Volume of Cellular Substance	
	Unexercised I—VI	Exercised VI	Unexercised I—VI	Exercised VI	Unexercised I—VI	Exercised VI
			Humerus			
Tangential and Transitional Zones.....	43.7±2.5	50.7±5.6	−161.9±14.7	−256.3±38.6	3456±1077	5328±2058
Transitional and Radial Zones	26.8±0.8	20.8±1.8	59.4±16.0	107.1±43.5	12073± 546	13184±1361
Tangential and Radial Zones	70.5±2.4	71.5±5.4	−102.5±12.3	−149.2±32.8	15529± 983	18512±1831
			Scapula			
Tangential and Transitional Zones.....	46.2±3.4	42.9±7.3	−49.5±11.4	−63.4±27.7	7384± 955	8059±2781
Transitional and Radial Zones	37.5±1.2	29.0±2.0	6.9±11.4	17.9±22.7	9844± 404	9083± 923
Tangential and Radial Zones	83.7±3.2	71.9±7.0	−42.6±10.6	−45.5±21.7	17228± 887	17142±2652
			Humerus and Scapula			
Tangential Zones.....	−8.0±4.0	3.2±8.8	32.2±10.6	60.5±26.5	1705±1297	5506±3133
Transitional Zones	−5.5±1.4	−4.6±2.5	144.6±15.3	253.4±39.6	5633± 622	8237±1464
Radial Zones	5.2±0.5	3.6±1.0	92.1±12.3	164.2±29.0	3404± 268	4136± 750

Summary

This investigation was carried out in order to study the manner in which the tissue of a normal, adult articular cartilage reacts when influenced by a physiologically increased function.

Approximately 140 male guinea-pigs were used in the experiments, which were started when the animals were 11 $^1/_2$ months old. The material was divided into unexercised animals and those for exercise, these main groups in their turn being divided into subgroups (Table 1, p. 21).

All the animals lived in ordinary cages of the same size. Those selected for exercise had to run 1,000 m per day in a special apparatus. After every 20th day of exercise one subgroup was killed, simultaneously with one that was not exercised.

After a histotechnical treatment of the shoulder-joints, the articular cartilages of the humerus and the scapula were examined microscopically within their weight-bearing parts. The three zones of the uncalcified cartilage, viz. the superficial *tangential*, the *transitional*, and the *radial* zone, were examined separately.

The differences observed between the various groups of the unexercised animals, as to the factors examined, could be ascribed to random variations. For this reason the control-animals were conjoined into a single group. Each exercised group was then compared with this control-material.

Results.

As to *the number of cells per unit volume of tissue*, it may be said that, in the transitional and the radial zones of the two cartilages, there was an increase of the number of cells in the beginning of the exercise-period (Group II), and then a rather successive decrease

in connection with continuous exercise, so that the last group exercised (Group VI) had a lower value than the control-material. In the tangential zones there occurred differences between the exercised groups and the control-material which were not statistically significant and arose more irregularly (Figs. 6 and 7).

The number of cells per chondron in the tangential and radial zones did not reveal any differences between the various exercised groups and the control-material, neither as to the humerus nor the scapula. In the transitional zones, on the contrary, there was found an increase of the number of cells per chondron in the exercised Groups II and V, and there was a certain parallelism between the number of cells per chondron and per unit volume (Fig. 8).

In the General Discussion it has been pointed out that there are reasons to assume the reproduction of cells in the cartilage as mainly occurring in the transitional zone. In the radial zone reproduction is probably present, though slight. The cells in the tangential zone seem chiefly to originate from those of the transitional zone through a secondary displacement. For, as a matter of fact, the decrease of the number of cells per unit volume and per chondron, concomitant to the continued exercise, appears to depend on a more marked formation of intercellular substance, whereby the cells are removed from each other.

The sizes of the cells in all the zones of the humeral cartilage grew larger during the course of the exercise. No corresponding increase could be determined regarding the scapular cartilage (Figs. 9 and 10).

The proportion between the cellular and the intercellular substance was temporarily changed in the transitional zones in favour of the cellular one. In the last group exercised, however, there was on the whole the same proportion as in the unexercised animals with respect to all the zones examined (Figs. 11 and 12).

The thickness of the uncalcified cartilage of the humerus was practically constant in the various exercised groups, and was the same as in the control-animals. The scapular cartilage showed a slight, statistically very probable, increase in the last group exercised. Yet, some reservations had to be made for this value (Tables 11 and 12).

Consequently, in this investigation it has been proved that *the tissue of an adult articular cartilage reacts on an increased functioning of a joint.* In the present study, this reaction appeared in varying degrees of structural modifications within the cartilage during the exercise, without definite changes in its thickness.

The results also *seem to elucidate the process of continuous regeneration* of the articular surface, the transitional zone playing a very prominent rôle as a centre of reproduction of cartilage cells. And, in consequence of the concomitant formation of intercellular substance, cartilaginous tissue is probably displaced from this zone towards the articular surface, whereby the products of wear and tear are compensated.

Finally, what has been ascertained concerning the transitional zone indicates that this zone must be left intact as far as possible if an appreciable repair of artificial defects in the articular cartilage is to be obtained.

References.

AUMONIER, F. J., J. Roy. Micr. Soc., *58*, 253, 1938.

BACKMAN, G., EDLÉN, Å., HJORTSJÖ, C.-H., and LÖFGREN, F., K. Fysiograf. Sällsk:s i Lund Förhandl., *18*, 24, 1948.

BAUER, W., BENNETT, G. A., MARBLE, A., and CLAFLIN, D., J. Exper. Med., *52*, 835, 1930.

BAUER, W., ROPES, M. W., and WAINE, H., Physiol. Rew., *20*, 272, 1940.

BENNETT, G. A., BAUER, W., and MADDOCK, S. J., Am. J. Path., *8*, 499, 1932.

BENNINGHOFF, A., Verhandl. d. anat. Gesellsch., *55*, 250, 1922.

——, Verhandl. d. anat. Gesellsch., *58*, 194, 1924.

——, Ztschr. f. d. ges. Anat., Abt. 1, *76*, 43, 1925.

——, Ztschr. f. d. ges. Anat., Abt. 3, *26*, 1, 1925.

——, Ztschr. f. Zellforsch. u. mikr. Anat., *2*, 783, 1925.

——, Lehrb. d. Anatomie des Menschen. Bd. I: Allgemeine Anatomie und Bewegungsapparat. München-Berlin 1939.

BOEHM, R., Beiträge zur normalen und pathologischen Anatomie der Gelenke. Inaug.-Diss. Würzburg 1868. Cit. Hammar.

BORMUTH, H., Ztschr. f. Zellforsch. u. mikr. Anat., *17*, 767, 1933.

BRINTON, W., Todd's Cyclopaedia, *4: 1*, 511, 1847—49.

BUCHER, O., Anat. Anz., *93*, 306, 1942.

CIOCIOLA, F., Policlinico (sez. chir.), *28*, 229, 1921. Ref. Zentralorg. f. d. ges. Chir., *14*, 125, 1921.

DAHLBERG, G., Statistical Methods for Medical and Biological Students. London 1940.

DEBRUNNER, H., Schweiz. med. Wchnschr., *6*, 692, 1925.

EKHOLM, R., To be published in Acta anat., 1951.

EKHOLM, R., and NORBÄCK, B., To be published in Acta orthop. Scandinav., 1951.

ELLIOT, H. C., Am. J. Anat., *58*, 127, 1936.

ELY, L. W., and MENSOR, M. C., Surg., Gynec. & Obst., *57*, 212, 1933.

FASOLI, G., Arch. per le sc. med., *29*, 365, 1905.

FICK, R., Handb. der Anatomie und Mechanik der Gelenke. Teil I—III. Jena 1904—11.

FISHER, A. G. T., Brit. J. Surg., *10*, 52, 1922—23.

——, Brit. Med. J., *2*, 390, 1939.

FISHER, R. A., Statistical Methods for Research Workers. London 1948.

FLODÉRUS, S., Acta path. et microbiol. Scandinav. Suppl. *53*, 1944.
FREUND, E., Arch. Surg., *39*, 596, 1939.
GIES, T., Deutsche Ztschr. f. Chir., *18*, 8, 1883.
GUSSENBAUER, C., Arch. f. klin. Chir., *12*, 791, 1871.
HÄBLER, C., Beitr. z. klin. Chir., *134*, 602, 1925.
HAMMAR, J. A., Bidrag till ledgångarnes histologi. Inaug.-Diss. Upsala 1892.
——, Arch. f. mikr. Anat. *43*, 266, 813, 1894.
HANSEN, FR. C. C., Anat. Hefte, *27*, 535, 1905.
HARRIS, H. A., Bone Growth in Health and Disease. Oxford medical publications, 1933.
HOLMDAHL, D. E., Nord. med., *14*, 1667, 1942.
——, Ztschr. f. Rheumaforsch., *5*, 320, 1942.
HOLMDAHL, D. E., and INGELMARK, B. E., Zool. bidrag fr. Uppsala, *25*, 91, 1947.
——, Acta anat., *6*, 309, 1948.
HULTÉN, O., and GELLERSTEDT, N., Acta chir. Scandinav., *84*, 1, 1940.
HULTKRANTZ, J. W., Das Ellenbogengelenk und seine Mechanik. Inaug.-Diss. Jena 1897.
——, Anat. Anz. Ergänzungsheft *14*, 248, 1898.
INGELMARK, B. E., Acta anat., *6*, 113, 193, 1948.
INGELMARK, B. E., and EKHOLM, R., Upsala läkaref. förh. [Acta Soc. med. Upsaliensis], *30: 4*, 61, 1948.
——, personal communication.
INGELMARK, B. E., and SÄÄF, J. E., Acta orthop. Scandinav., *17*, 303, 1948.
ITO, L. K., Brit. J. Surg., *12*, 31, 1924—25.
JAFFÉ, R., Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere. Berlin 1931.
JOHN, K., Ztschr. f. wissensch. Mikr., *46*, 395, 1929—30.
JUNGHANS, H., Der Stütz- und Bewegungsapparat. Leipzig 1939.
KEY, J. A., Anat. Rec., *40*, 193, 1928.
KJELLGREN, K., Studien über die Entwicklung der Neuronen nach der Geburt, ihre Regeneration und die Asymmetrien ihrer Verteilung beim Menschen. Inaug.-Diss. Uppsala 1944.
KOCH, H., Deutsche Ztschr. f. Chir., *201*, 367, 1927.
LUBOSCH, W., Bau und Entstehung der Wirbeltiergelenke. Jena 1910.
——, Ztschr. f. mikr.-anat. Forsch., *11*, 67, 1927.
——, Handb. d. vergl. Anat. der Wirbeltiere, *5*, 249, 305, 1938.
MARCHAND, F., Deutsche Chir., *16*, 268, 1901.
MATSUOKA, M., Virchows Arch. f. path. Anat., *175*, 32, 1904.
MOLL, A., Virchows Arch. f. path. Anat., *105*, 466, 1886.
MORI, M., Deutsche Ztschr. f. Chir., *76*, 220, 1905.
MÜLLER, W., Ztschr. f. orthop. Chir., *44*, 478, 1923—24.
——, Beitr. z. klin. Chir., *131*, 642, 1924.
——, Biologie der Gelenke. Leipzig 1929.
——, Jahresk. f. ärztl. Fortbild., *21: 1*, 29, 1930.

NATHAN, P. W., and STRANG, W. W., Am. J. Orthop. Surg., *7*, 85, 1909—10.
OGSTON, A., J. Anat. Physiol., *10*, 49, 1876.
PAYR, E., Deutsche med. Wchnschr., *44: 2*, 817, 844, 874, 1918.
RAEBIGER, H., Das Meerschweinchen, seine Zucht, Haltung und Krankheiten. Hannover 1923.
RETTERER, E., Soc. de biol., *64*, 45, 117, 155, 1908.
REYHER, C., Deutsche Ztschr. f. Chir., *3*, 189, 1873.
ROUX, W., Gesammelte Abhandlungen über Entwicklungsmechanik der Organismen. I. Funktionelle Anpassung. II. Entwicklungsmechanik des Embryo. Leipzig 1895.
RUPPRICHT, W., Arch. f. mikr. Anat., *75*, 748, 1910.
SCAGLIETTI, O., Ztschr. f. orthop. Chir., *52*, 577, 1929—30.
SCHAFFER, J., Handb. d. mikr. Anat. des Menschen von W. von Möllendorff, *2*, 210, 1930.
SEGGEL, R., Deutsche Ztschr. f. Chir., *75*, 453, 1904.
SEKI, M., Ztschr. f. Zellforsch. u. mikr. Anat., *26*, 305, 1937.
SHANDS, A. R., Arch. Surg., *22*, 137, 1931.
SILBERBERG, M., New York Proc. Soc. Exper. Biol. Med., *34*, 333, 1936.
SILBERBERG, M., and SILBERBERG, R., Arch. Path., *26*, 1208, 1938.
——, Am. J. Path., *15*, 55, 547, 1939.
——, Anat. Rec., *78*, 549, 1940.
——, Arch. Path., *29*, 355, 1940.
——, Growth, *4*, 305, 1940.
——, Am. J. Anat., *69*, 295, 1941.
——, Arch. Path., *31*, 85, 1941.
——, Am. J. Path., *17. 1*, 189, 1941.
——, Arch. Path., *32*, 85, 1941.
——, Endocrinology, *31*, 410, 1942.
STOWELL, R. E., Stain Technol., *16*, 67, 1941.
SUGITA, N., J. Comp. Neurol., *28*, 511, 1917.
SÄÄF, J. E., Upsala läkaref. förh. N. F. [Acta Soc. med. Upsaliensis], *46*, 349, 1941.
TARKHAN, A. A., J. Roy. Micr. Soc., *51*, 387, 1931.
TELLYESNICZKY, K., Arch. f. mikr. Anat., *52*, 202, 1898.
TILLMANNS, H., Arch. f. mikr. Anat., *10*, 401, 1874.
——, Arch. f. Anat. u. Entwcklgsgesch., p. 2, 1877.
TIZZONI, G., Arch. per le sc. med., *2*, 28, 1878. Cit. Shands.
WEHNER, E., Deutsche Ztschr. f. Chir., *180*, 201, 1923.
WEICHSELBAUM, A., Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. kl., Abt. 3, *75*, 193, 1877.
WERNER, H., Die Dicke der menschlichen Gelenkknorpel. Inaug.-Diss. Berlin 1897.