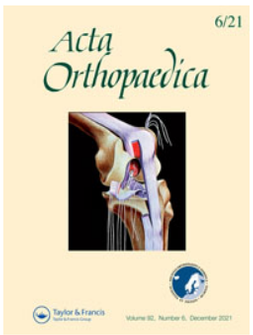

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JOINT CHANGES AFTER OVERUSE AND PEAK OVERLOADING OF RABBIT KNEES *IN VIVO*

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The effect of overuse and overuse combined with axial peak overloading on the knee joints of living rabbits has been investigated. A specially constructed apparatus was used for this purpose. Physical and biochemical changes are reported and include:

1. Early and progressive damage to the articular cartilage surface shown by the scanning electron microscope.
2. The presence of an increased amount of prostaglandin E in the synovial fluid.
3. A reduction of cyclic 3′–5′ adenosine monophosphate in the subchondral bone.
4. Late changes which were consistent with osteoarthritis.

These changes were found only in the joints subjected to simultaneous overuse and peak overloading.

The results suggest that:

1. Cartilage damage and chemical changes in the subchondral bone are simultaneous and are both responsible for eventual degenerative changes.
2. Frictional overuse alone does not seem to be responsible for the production of osteoarthritis.

Key words: Cyclic 3′–5′ adenosine monophosphate; overuse; peak overloading; prostaglandin E; scanning electron microscope

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Weight-bearing joints are subjected to two kinds of stress. One is a shear stress produced by reciprocal friction of the articulating surfaces and the other is produced by axial longitudinal loading. Clinical conditions in which either of these stresses is abnormally increased may induce degenerative changes.

Accordingly, experiments designed to reproduce such changes have involved either modifications of the articular surfaces by chemical or mechanical means (Bennet & Bauer 1937, Trias 1961, Crellin & Southwick 1964, Murray 1964, Hulth et al. 1970) or by increasing the axial longitudinal loading (Simon et al. 1972, Radin et al. 1973).

However, *in vivo*, both stresses are simultaneous and cannot be separated. It was thought important, therefore, to explore this simultaneous action in the living animal under controlled conditions which allow the separation of these effects.

The present study reports the physical and biochemical changes found in knees of living rabbits after these joints were subjected simultaneously to frictional and loading stress by means of a specially constructed apparatus. One joint was subjected to both stresses. The contralateral one, serving as a control, was subjected to frictional stress only.

EXPERIMENTAL PROCEDURE

White New Zealand adult female rabbits weighing 3–4 kg were used. Skeletal maturity as evidenced by the closure of the lower epiphysis of the femur and the upper epiphysis of the tibia was ascertained roentgenographically. Each rabbit was anaesthetized by open mask Fluothane (ICI) and maintained by the addition of 10 mg/kg of Nembutal (Abbott Laboratories). Under sterile conditions, incisions were made both on the lateral and medial aspects of the thigh and the leg, 3 cm away from the knee joint line. Through these incisions two parallel round stainless steel pins were introduced, one into each bone with the aid of a low speed dental handpiece drill. The pin used for the femur was 2.4 mm in diameter and that for the tibia was 2.9 mm (Stainless steel Zimmer, USA.). The skin was then closed by silk sutures. A small pad with Betadine was applied around the protruding pins.

The animal was then tied to a specially constructed apparatus. This consisted of a platform (on which the animal could rest with its legs hanging down) and an electric motor (60 watt) which moved a wheel. The upper two pins, one for each femur, were secured to the body of the apparatus. The legs and the feet were connected to the driving-wheel by means of a flexible rod. Because of the eccentric attachment of the rod, the rotation of the wheel produced alternative flexion and extension of the knees (Figure 1).

Each revolution of the wheel moved the knee joints from flexion of 80° to full extension. A forked device which was connected to the driving-wheel produced direct impulsive peak overloads to the pin of one of the tibiae. This load was adjusted by a spring to a maximum of 350 g. Therefore, each revolution of the driving-wheel caused:

(a) movement of the two knees from 80° flexion to full extension, and
(b) at the point of full extension of both knees peak overloading was applied to the left leg.

Because of the rigid fixation of the bone to the apparatus, the peak loads exerted on the knee joints were direct and bone conducted. Furthermore, the constant distance of the pins from the knee joint enabled identical experimental conditions regardless of the size of the animals' legs. The forces applied to the joints, either in the number of revolutions or the number of peak loadings, must be considered as being above the normal. Consequently, the abnormal number of flexion/extension movements is defined as overuse and the axial loadings as peak overloading.

Figure 1. Left: The mode of fixation of the animal on the specially constructed apparatus. Upper right: Flexion of the knee. Lower right: Extension of the knee with the mode of the delivered impact.

MATERIAL AND METHODS

These are summarized in Table 1. Forty-two rabbits were divided into seven groups. The rabbits of Groups A, B and C were subjected to 30, 60 and 120 minutes at 60 revolutions per minute of the driving-wheel (1800, 3600 and 7200 flexions and peak loadings in all). At the end of each of the experiments the animals of these groups were killed immediately and the articular surfaces of the knee joints were examined by scanning electron microscopy (SEM) and biochemically. In the three rabbits of Group D (7200 revolutions), only the articular cartilage, menisci and synovial membrane were examined for their prostaglandin/(PGE) content, immediately after completion of the experiment. The three rabbits of Group E were subjected to the same experimental procedure as those of Group C but were killed 1, 3 and 7 days after the completion of the experiment. Only SEM of the articular cartilage surface was done in this group.

For the scanning electron microscope examination, the articular surface was immediately dissected and fixed at the end of the experiment. A critical point drying was done and then it was given conductive coats of carbon and gold according to a method described by Boyde & Jones (1974). The specimens were examined and photographed in a Cambridge Steroscan 180 scanning electron microscope. The pictures of both knees were compared.

The biochemical examinations included (a) analyses for prostaglandin E group (PGE) in the

Table 1. Summary of the investigation

Group	No. of rabbits	Duration of experiment (minutes)	No. of revolutions	Examination performed	Biochemical examination			Microscopical and X-ray examination		
					cAMP in bone and cartilage	PGE in synovial fluid	PGE in cartilage, menisci and synovia	SEM	Histology	X-ray
A	8	30	1800	Immediate	+ (5 rabbits)	+ (5 rabbits)	–	+ (3 rabbits)	–	–
B	8	60	3600	Immediate	+ (5 rabbits)	+ (5 rabbits)	–	+ (3 rabbits)	–	–
C	8	120	7200	Immediate	+ (5 rabbits)	+ (5 rabbits)	–	+ (3 rabbits)	–	–
D	3	120	7200	Immediate	–	–	+ (3 rabbits)	–	–	–
E	3	120	7200	After 1, 3, 7 days	–	–	–	+ (3 rabbits)	–	–
F	6	30 min/day for 5 days	9000	After 75 days	–	–	–	–	+ (6 rabbits)	+ (6 rabbits)
G	6	30 min/day for 15 days	27000	After 85 days	–	–	–	–	+ (6 rabbits)	+ (6 rabbits)

synovial fluid, the articular cartilage, the menisci and the synovial membrane and (b) the estimation of cyclic 3′, 5′ adenosine monophosphate (cAMP) in the bones and cartilage. For the former, the knee joints were dissected at the end of the experiments, the synovial spaces were rinsed with 0.5 cm^3 of cold 50 mM tris buffer at pH 7.0 and analysed for PGE content by radio-immuno assay (Bauminger et al. 1973). The articular cartilage, menisci and synovial membrane obtained from the three rabbits of Group D were homogenized in 50 mM tris EDTA buffer pH 7.0 and analysed for PGE content by the same method.

For the estimation of cAMP, the bones on each side of the knee joints were immediately frozen in liquid nitrogen and cut transversely into 3 mm thick samples and pulverized in liquid nitrogen by a metal piston. After adding the bone powder to distilled water, the mixture was boiled for 5 min in a salt bath (Shanfeld et al. 1975). The samples were then further homogenized by an ultrathorax (Tp 18–10).

The cAMP content was estimated in the supernatant by the protein binding assay (Gilman 1970). As the samples from each rabbit were treated identically, the results obtained from the overuse and peak overloaded bones were compared with those obtained from the contralateral bones subjected to overuse only and expressed as the percentage of change.

The rabbits in the groups F and G (six rabbits in each group) served for the estimation of the late changes in the articular cartilage and the bones. In these groups the experiment was run for a period of 30 min at 60 rev/min of the driving-wheel, each day for 5 and 15 days, 9000 and 27000 revolutions, respectively. In between the experiments the rabbits were allowed to move freely in the cages. Care was taken to protect the protruding pins. Two separate small bandages were applied on each leg in such a way as to allow free movements of the joints. At the end of the experimental period the pins were removed and 10 weeks later, the animals were killed. High resolution roentgenograms of the knee joint were taken, and thereafter the joint was examined histologically. After decalcification a sagittal section of the joint was done, and stained by haematoxylin eosin.

RESULTS

Scanning electron microscopy (SEM)

The changes in the morphology of the articular surface seen in the knees which were subjected to simultaneous overuse and peak overloading consisted of damage to the superficial layer, which was found to be detached exposing the collagenous matrix. These changes occurred only when the experiment lasted at least 1 hour (3,600 revolutions and loadings). At that time only a limited area was affected (Figure 2). The affected area was more extensive when the experiment lasted 2 hours (7200 revolutions and loadings) (Figures 3, 4). The damage was even more pronounced when the knee was subjected to 7200 revolutions and loadings and examined after 3 or 7 days (Figure 5).

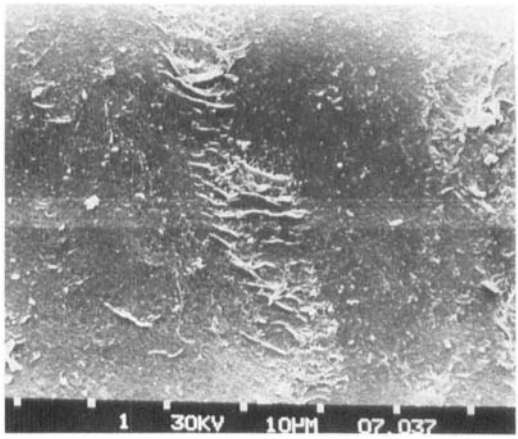

Figure 2. SEM of articular surface of a femoral condyle after 1 hour of overuse and peak overloading showing both undamaged regions and damaged ones containing debris (× 1680).

No such changes were seen in the contralateral joint submitted to overuse only (Figure 6).

It may therefore be concluded that peak overloading had long-lasting and progressive physical effects, the initial damage giving but an incomplete picture of the later deterioration of the joint surface.

Prostaglandin E group (PGE)

When the knees subjected to simultaneous overuse and peak overloading were examined after 2 hours of exercise (7,200 revolutions and loadings), the synovial fluid was found to

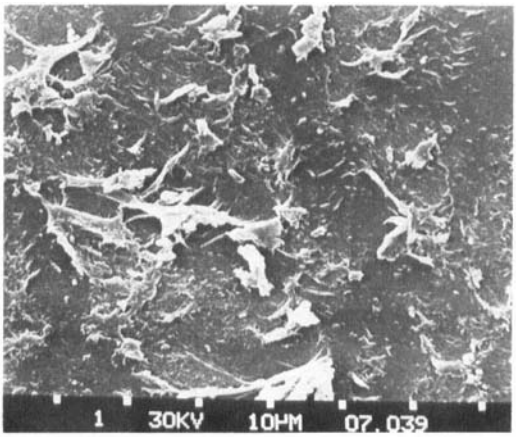

Figure 3. SEM of articular surfaces of a femoral condyle after 2 hours of overuse and peak overloading showing damage and abundant debris (× 1500).

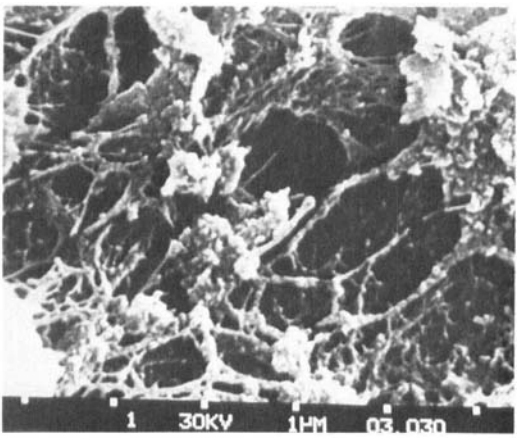

Figure 5. SEM of articular surface taken 7 days after 2 hours of overuse and peak overloading. The cellular layer of the articular surface is detached and the collagenous matrix is exposed. Empty lacunae can be seen (× 15,000).

contain a five-fold increase in prostaglandin E, as compared with the contralateral knee (Figure 7). No significant increase of PGE was found after 30 or 60 min of exercise (1800 and 3200 revolutions and loadings). No significant changes in the amount of prostaglandin were found in the synovial membrane, the articular cartilage or the menisci of both knees.

Cyclic 3'–5' Adenosine Monophosphate (cAMP)

The knees subjected to simultaneous overuse and peak overloading showed a reduction of the cAMP content after 60 min of overuse and peak overloading (3600 revolutions), as compared with the other knee. The reduction was more pronounced in

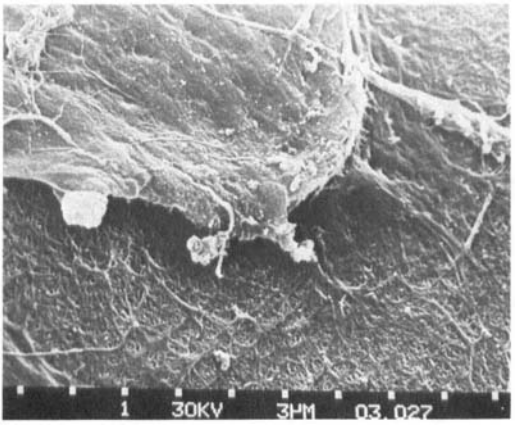

Figure 4. SEM of articular surface of a femoral condyle after 2 hours of overuse and peak overloading showing detail of a semi-detached piece of debris (× 10,000).

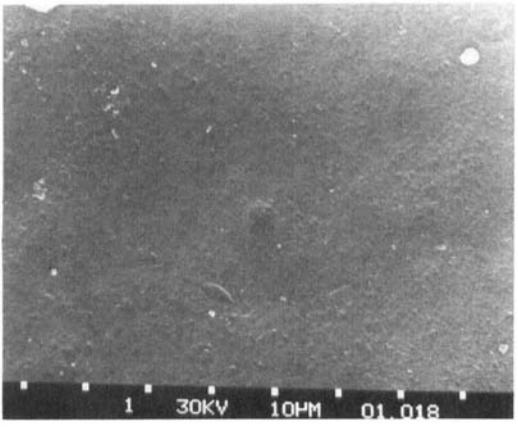

Figure 6. SEM of articular surface of a femoral condyle after 2 hours of overuse only, showing a smooth undamaged surface (× 1500).

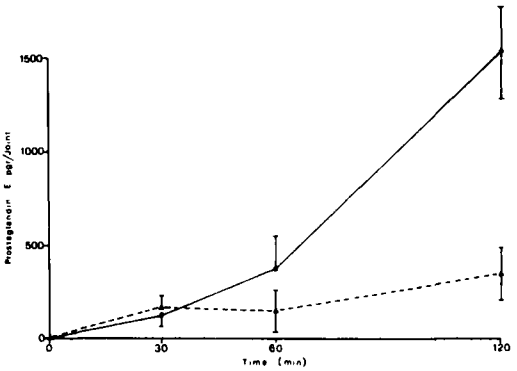

Figure 7. Accumulation of prostaglandins E series in the synovial fluid in overused knees (broken line) and overused and peak-overloaded knees (continuous line).

the subchondral bone and occurred first in the tibia and later in the femur. There were no significant changes, regardless of the number of revolutions, either in the articular cartilage or in the cortical bone (Table 2).

X-ray

Flattening of the epiphysis, small bone cysts and osteophytes were found on the roentgenograms of knees subjected to repeated simultaneous overuse and peak overloading (1800 revolutions and loadings per day for 15 consecutive days) (Figure 8). Milder changes were seen when the experiments were repeated for 5 days only. No such abnormality was found in the contralateral knees subjected to overuse only.

Histology

Repeated simultaneous overuse and peak overloading (1800 revolutions and loadings per day for 15 days) resulted in cellular degeneration, disturbed arrangement of the cells with cluster formation and fibrillation of the articular cartilage with penetration of the subchondral capillaries into the calcified layer of the cartilage. There was thickening of the subchondral bone with the presence of

Table 2. The cAMP content in different anatomical areas of the knee. The percentages have been calculated according to the following formula:

$$\frac{cAMP\ left\ knee - cAMP\ right\ knee}{cAMP\ right\ knee} \times 100$$

(right knee = overuse only)
(left knee = overuse and peak overloading)

THE cAMP CONTENT OF ARTICULAR CARTILAGE, SUBCHONDRAL, SPONGIOUS AND CORTICAL BONE

Duration of the experiment	30 min	60 min	120 min
	$\bar{X}$ ± SE	$\bar{X}$ ± SE	$\bar{X}$ ± SE
Cortical bone	- 0.93% ± 8.87	-13.45% ± 11.11	- 9.84% ± 10.41
Spongious bone	- 2.92% ± 9.04	- 2.69% ± 9.04	-32.0% ± 12.44•
Subchondral bone	+ 3.25% ± 8.65	-12.84% ± 3.96•	-36.0% ± 2.29•
Cartilage	+ 4.10% ± 10.88	- 0.43% ± 14.29	- 1.25% ± 9.37
Cartilage	- 1.90% ± 7.95	- 7.01% ± 7.45	+ 7.25% ± 22.30
Subchondral bone	+ 0.06% ± 6.45	-42.9 % ± 3.12•	-49.33 ± 6.32•
Spongious bone	+ 3.81% ± 11.85	-33.52% ± 7.51•	-25.13 ± 4.69•
Cortical bone	- 2.93% ± 8.72	+ 8.06% ± 9.23	+ 3.04 ± 9.04

•P < 0.05

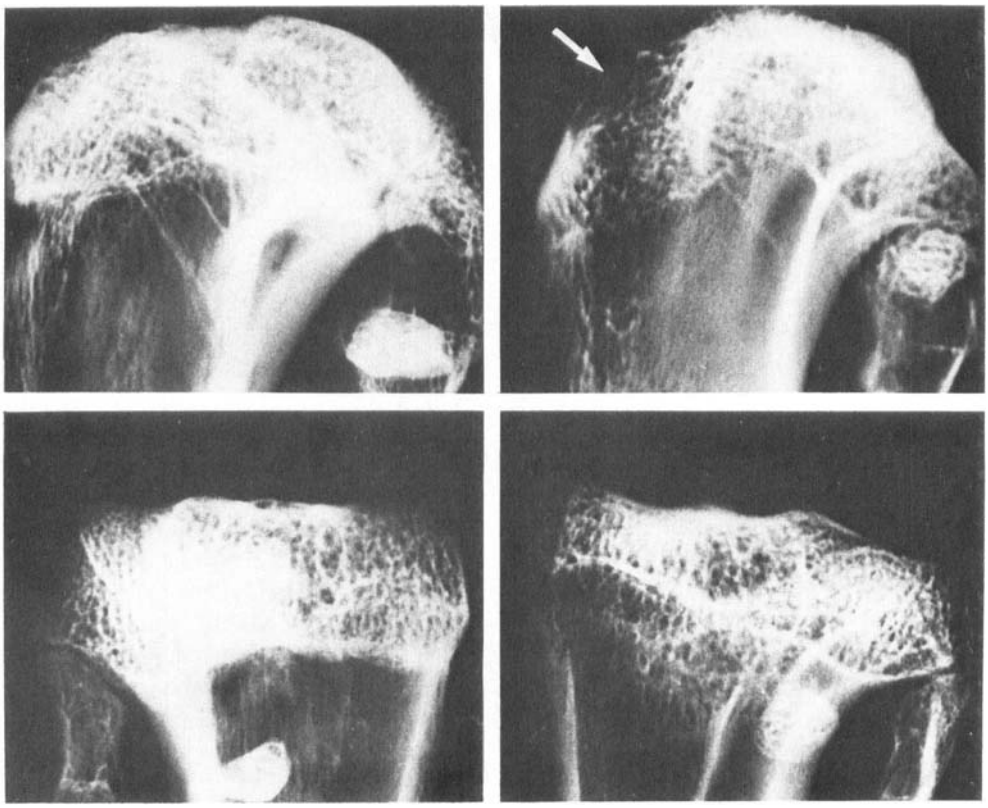

Figure 8. Roentgenograms taken 10 weeks after 15 consecutive days of overuse (at left) and overuse and peak overloading (at right) of the knee. The upper end of the tibia shows flattening of the medial part of the epiphysis with condensation of subchondral bone and cyst formation (arrow) in the peak overloaded knee.

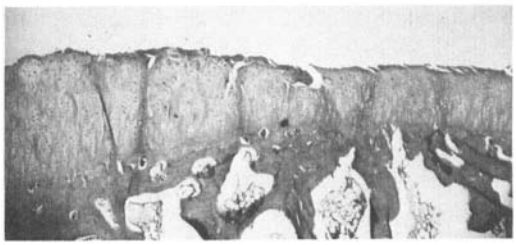

Figure 9. Sagittal section of a femoral condyle 10 weeks after 15 consecutive days of overuse and peak overloading showing fibrillated articular cartilage which becomes progressively thinner from left to right. The tidemark has advanced into the mid-zone of the cartilage and there is increased subchondral vascularization (× 80)

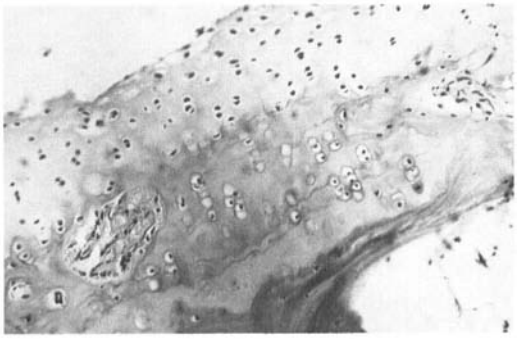

Figure 10. Sagittal section of a femoral condyle 10 weeks after 15 consecutive days of overuse and peak overloading showing disorganization of cartilage cells and hypocellularity in the radial zone with empty lacuna. The tidemark is broken and penetrated by blood vessels from subchondral bone. The subchondral bone is thick and new lamellar bone has formed (× 300).

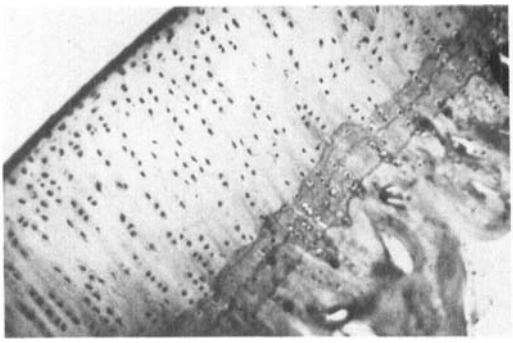

Figure 11. Articular cartilage from an overused knee 10 weeks after 15 days of exercise showing a normal arrangement of the cartilage in the various zones. The tidemark is intact and there are no changes in the subchondral bone (×300).

lamellar bone parallel to the articular surface (Figures 9, 10). These changes, consistent with the pattern of osteoarthritis, were not found in the contralateral joint subjected to overuse only (Figure 11).

DISCUSSION

The fact that the observed changes occurred in knees subjected to simultaneous overuse and peak overloading but not in those subjected to overuse only permits the conclusion that it is the added peak overloading which is responsible for their occurrence. These changes are twofold: early physical and biochemical changes in the articular cartilage, the subchondral bone and synovial fluid and late changes in the articular cartilage and subchondral bone, consistent with osteoarthritis.

With regard to the physical changes in the articular cartilage, there are three points which merit some emphasis:—

1. The existence of a threshold below which no changes occur.
2. The direct relation of the extent of damage to the duration of the insult.
3. The progressive deterioration after cessation of the insult. It may be presumed that the last two in particular may have an influence on the appearance of the late osteoarthritic changes.

Increased amounts of PGE have been found in the synovial fluid of patients with septic arthritis and active rheumatoid arthritis (Velo. et al. 1973, Robinson & Levine 1974). Synovial cells derived from rheumatoid patients synthetize large quantities of PGE *in vitro* (Robinson et al. 1975b, Dayer et al. 1977). The high levels of prostaglandins in the joints of such patients are believed to be one of the factors leading to cartilage destruction and bone resorption (Eisenbarth & Lebovitz 1974, Robinson et al. 1975a, Dietrich & Raisz 1975). However the role of prostaglandins in osteoarthritis is not yet established. Our results may suggest that prostaglandins have an important role in the pathogenesis of osteoarthritis as a result of repetitive overloading. Furthermore the relative effectiveness in patients with osteoarthritis of anti-inflammatory drugs such as aspirin and indomethacin, which are potent prostaglandin synthetase inhibitors (Vane 1971, Ferreira et al. 1971), can be explained on this basis.

The changes in cAMP content in the subchondral bone are more difficult to interpret. Cyclic cAMP is thought to be the second messenger in regulating intracellular function as a consequence of the action of external stimuli, hormones in particular (Sutherland et al. 1965). Lately it was suggested that cAMP may act as a mediator of the mechanical stimuli in bone and cartilage (Rodan et al. 1975, Davidovitch & Shanfield 1976, Harell et al. 1977). In addition intimate correlation exists between cAMP levels and proliferate activity of various cell types. Reduction in cAMP concentration was observed during mitotic activity of fibroblasts, bone marrow and other cells (Otten et al. 1971, Tisman & Herbert 1973, Oler et al. 1973, Tell et al. 1973). The reduction of the cAMP content found in the subchondral bone may express either a reaction to the mechanical stimuli or an increased mitotic activity. However this reduction was specifically in the subchondral bone and not in the cartilage or cortical bone.

This observation confirms the concept developed by Radin et al. (1972, 1973) in which impact loading produced rapid changes in the subchondral bone, leading to bone stiffening through microfractures or bone resorption and new bone formation (Pugh et al. 1974). This subchondral remodelling is assumed to initiate secondary changes in the articular cartilage (Johnson 1962).

The results of our experiments do not allow us to draw definite conclusions as to the initial factor. It seems reasonable to assume that the damage to the cartilagenous surface and the biochemical changes in the subchondral bone may be simultaneous and that their cumulative effects lead to late osteoarthritic changes such as those seen in the groups of animals killed some time after completion of the experiments.

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