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CLINICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES ON ARTHRITIS IN EQUINE

BY

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SUMMARY

Expermintally chemically induced arthirtis was performed using single intraarticular injection of 10mg amphotricine B in left radiocarpal joint of 8 horses; 7 donkeys and 5 mules with normal joints. Clinical examination; radiological examination; synovial fluid analysis and histopathological examination were monitored over a period of 24 weeks. According to the results of the above mentioned parameters, degenerative joint disease can be classified into 2 main phases; Acute inflammatory and degenerative phase. The degenerative phase was into 3 stages. mild; moderate and severe.

INTRODUCTION

Literature on degenerative arthritis gives incomplete information concerning the correlation between different sages of degenerative arthritis and clinical, radiological, synovial and histopathological changes, its early diagnosis, and proper time of treatment.

Several methods for induction of experimental degenerative arthritis have been described by different authors. Chemical induction of osteoarthritis was reported by Axhausen (1910) and Burchhardt (1924) who produced experimental arthritis in knee joints in series of animals by application of tincture of iodine or amonium hydroxide. Seeliger (1926) and Key (1932) induced arthritis-like changes in the knee joints of rabbits by repeated intraarticular injections of HCI. Similar results were seen after repeated injection of distilled water (Halber, 1928). Nonspecific substances such as citrated blood or Indian ink can also produce arthritis (Key, 1930). weissman et al., (1965)caused acute and chronic arthritis in rabbits by intraarticular injections of streptolysine. The long term use of corticosteroids led to undetectable clinical signs of arthritis (steroid induced arthropathy) (Roach et. al., Leqinson et. al., 1980 and Shehab, 1988). Mcllwraith et. al., (1979) A&B) noted that the intraarticular injection of 400 µg of the polyene antibiotic filipin in mature ponies resulted in stifiness and thickening of the joint. Arthritis was also induced by intraarticular injection of 10mg of amphotricine B (Edwards et al., 1977 and Bowman et. al., (1983). The aim of this study is to obtain this study is to obtain the best animal model for studying degenerative arthritis.

MATERIAL AND METHODS

This study was carried out on 8 horses, 7 donkeys and 5 mules with nomal joints that were carefiully to be free of disease.

Arthiritis was induced by injecting 10 mg amphotericine B (Bowman et al., 1983) into radiocaypal joint. 5% dextrose solution was injected into the right carpal joint in order to serve as a control. The animals were subjected to daily clinical examination during the entire experimetal

period.

Radiographs were made of each carpus at the beginnign of the experiment, at weekly intervals and immediately prior to euthanasis. Synovial analysis was performed weekly till the end of the experiment (24 weeks). Samples of synovial fluid were obtained aseptically by arthrocentesis (Edwards et. al., 1977 and Rose et. al., 1982). The synovial fluid samples were transferred to plain and EDTA capped vials for examination. the laboratory and cytologyical values of the synovial fluid were evaluated at the time of arthrocentesis using the method described by VanPelt and Connor (1963). Biochemical analysis of synovial fluid was performed throughout the experimetla period. Total protein (Peterst 1968), alkaline phosphatse (Sommer, 1954), glutamic oxalacetic transarminase and glutamic pyruvic transaminase (Reitman et. al., 1957), lacitc acid dehydrogenase (Anon, 1970), lysozyme (Shugar, 1952) were measured. In adition, hyaluronic acid levels (Meyere et. al., 1960 adn tolksdorf et. al., 1979) were determined. Synovial samples were cultured and examined (Haupt, 1964).

Morphological and histopathological examination of the articular cartilage, subchondral bone, joint capsule and synovial membrane were performed after euthanasia. Bone samples were decalcified using formic acid/Hcl 10%. All sections were stained with haematoxyline and eosin using the method described by Carlton et. al., 1967.

RESULTS

Clinical signs of experimentally induced arthritis:

The clinical symptoms of amphotricine B injection were the same in all injected animals (Horses, Mules and Donkeys).

The early signs of the intra-articular injection were seen after 2-3 hours post injection. Lameness could be observed in the injected limb and reached its peak after 6 hours following injection. The animal could not bear its weight on the injected leg. This marked degree of lameness persisted for about 72 hours and then declined until it was unremarkable after 3 weeks, where it could be detected by palpation or pa movement of the joint.

Associated to lameness, acute swelling developed in and around the joints (Fig. 1). T joint capsule was distended due to syno effusion which persisted till euthanasia of animals. Chroniv thickening of the joint cap was also developed over the 7th week of induction period. There was a defin enlargement in circumference of the joint wit average of 1.5cm (+0.08) at the 24th week. T joint was severely hot after 6 hours and t returned to normality after 7 days.

Radiological findings:

The radiologicla abnormalities of the injec carpus were not observed up to the 7th week. the 8th week, periosteal reaction in the area carpal capsule on the dorsal surface of the ra carpal joints was observed (Fig. 2). The periost reaction in the area of the joint attachment on dorsal surface of carpoulnar bone were obser with progressing of the disease. At the 24th we osteophytes were observed on the medial side the joint at the mediodistal margin of the ra bone and/or the proximal margin of the carp ulnar bone (Fig. 3)

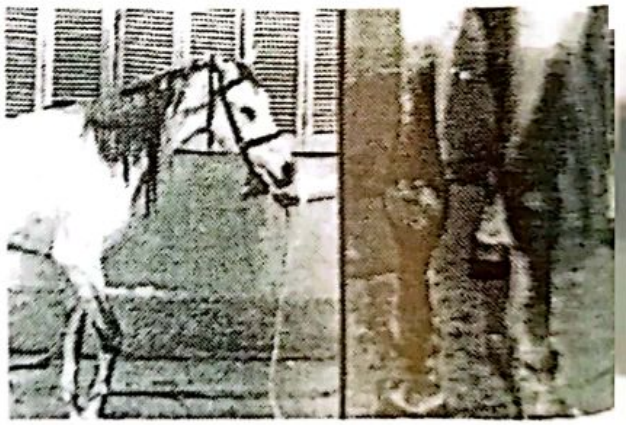
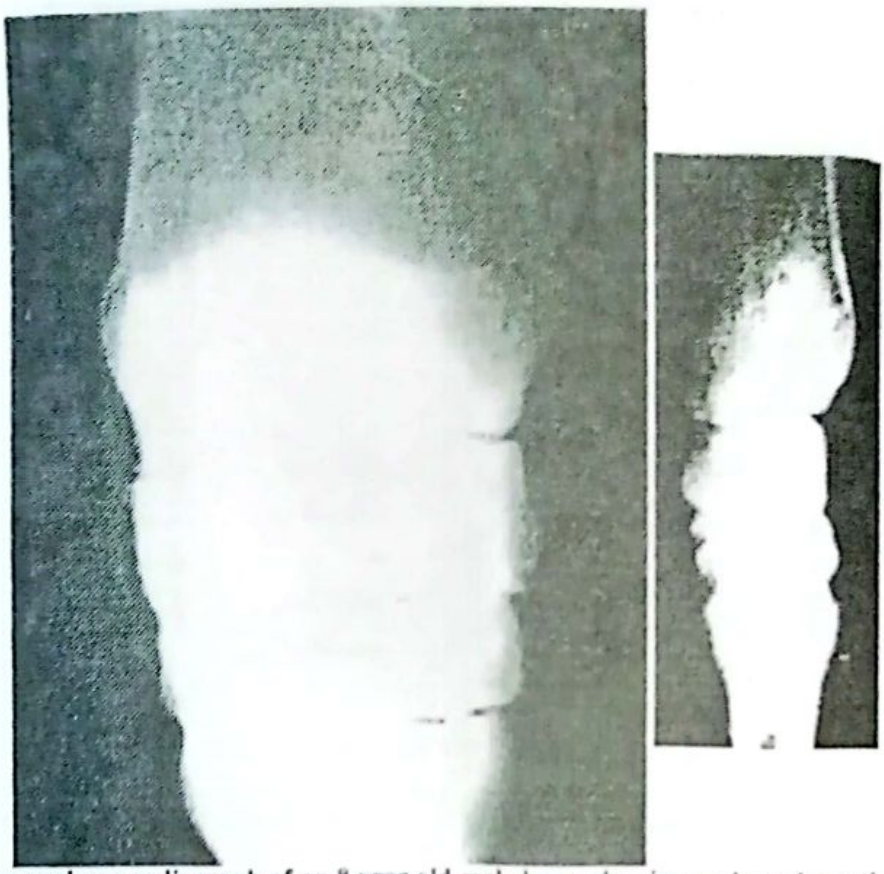
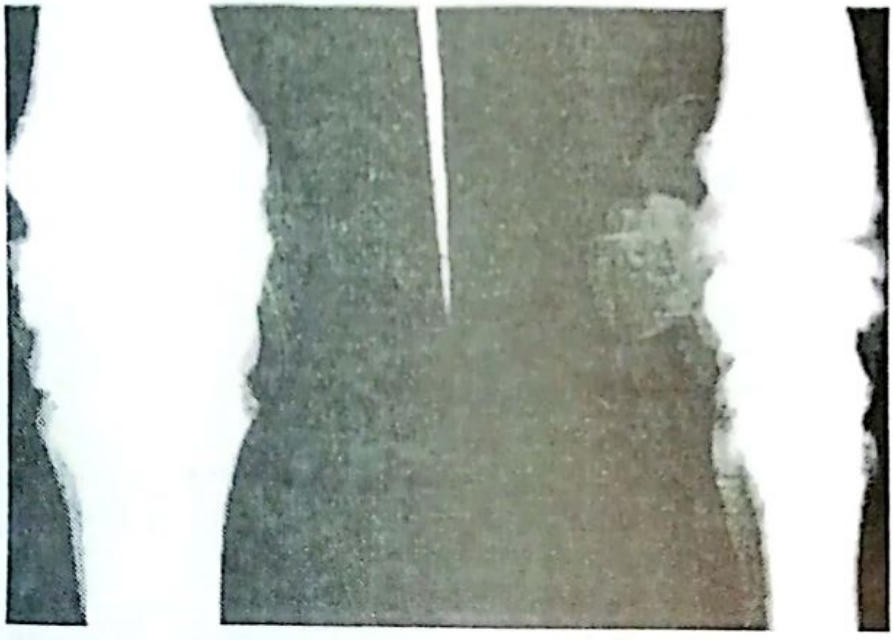


Fig. 1: Eight-year-old male horse showing swelling the left carpal joint after the injection of amph tricin B in comparison with right carpus wh was injected with dextrose 5% as a control.



2A: Dorsopalmar radiograph of an 8 yrar old male horse showing periosteal reaction on the dorsla surface of the radiocarpal joint after 8 weeks (arrow).
ateromedial new of rarpus showing soft tissue swelling on the dorsal swface of the carpus.



(A) lateromedial and (B) dorsopalmar radiographs of the radiocarpal joint of an 11 year old male horse showing norrowing of the radiocarpal joint space and subchondral bone sclerosis with marked osteoperistial reaction on the proximal surface of the carpal bone and distal end of the radius (arrow) soft tissue swelling in carpal region in also showed (24 weeks).

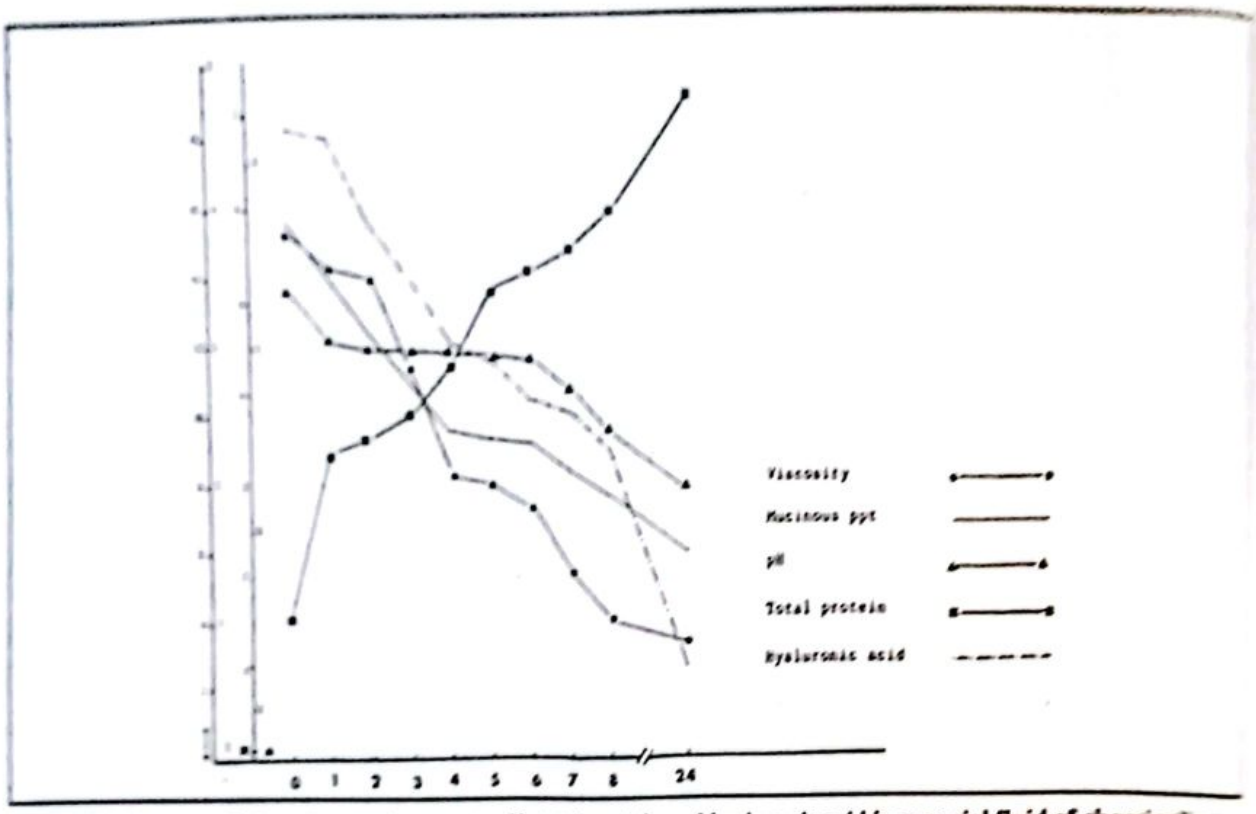


Fig. 4: The changes of viscosity, mucinous ppt, pH, total protein and hyaluronic acid in synovial fluid of chemically induced arthritis.

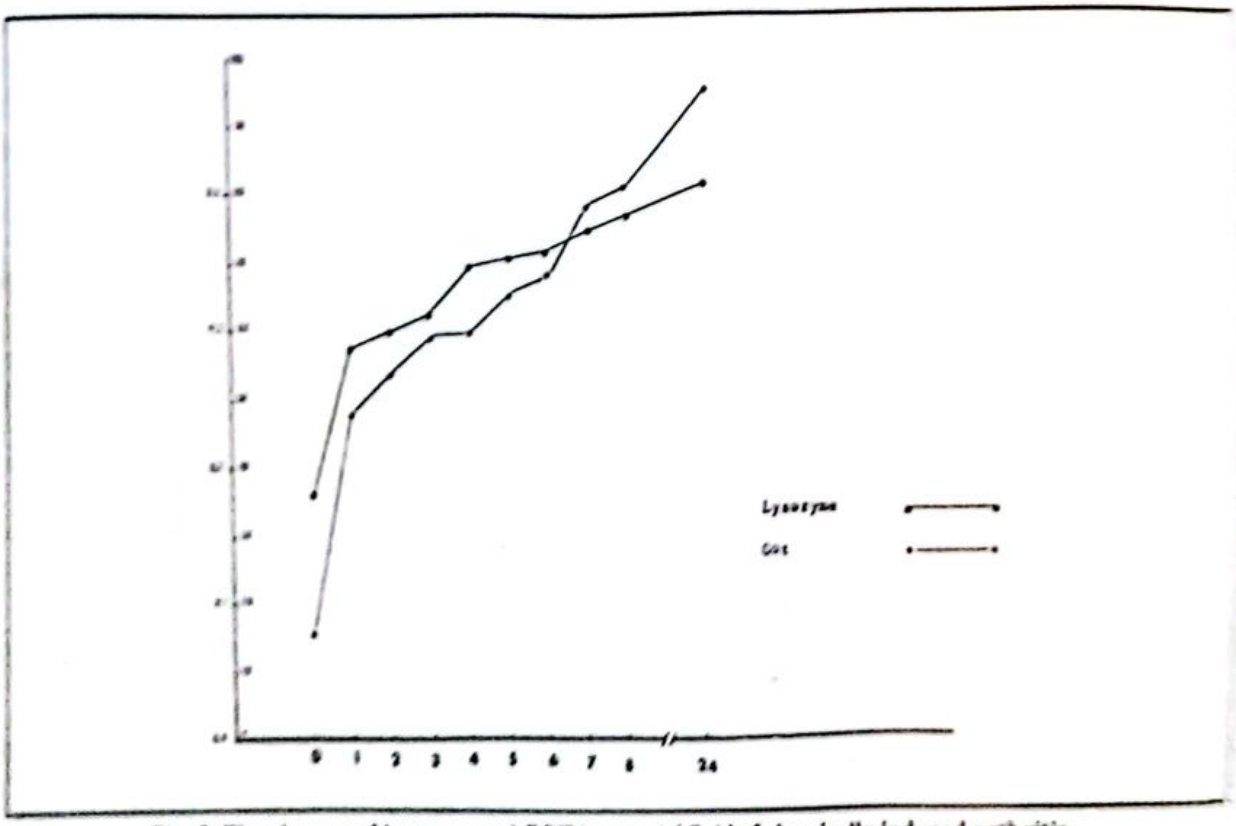


Fig. 5: The changes of lysozyme and GOT in synovial fluid of chemically induced arthritis.

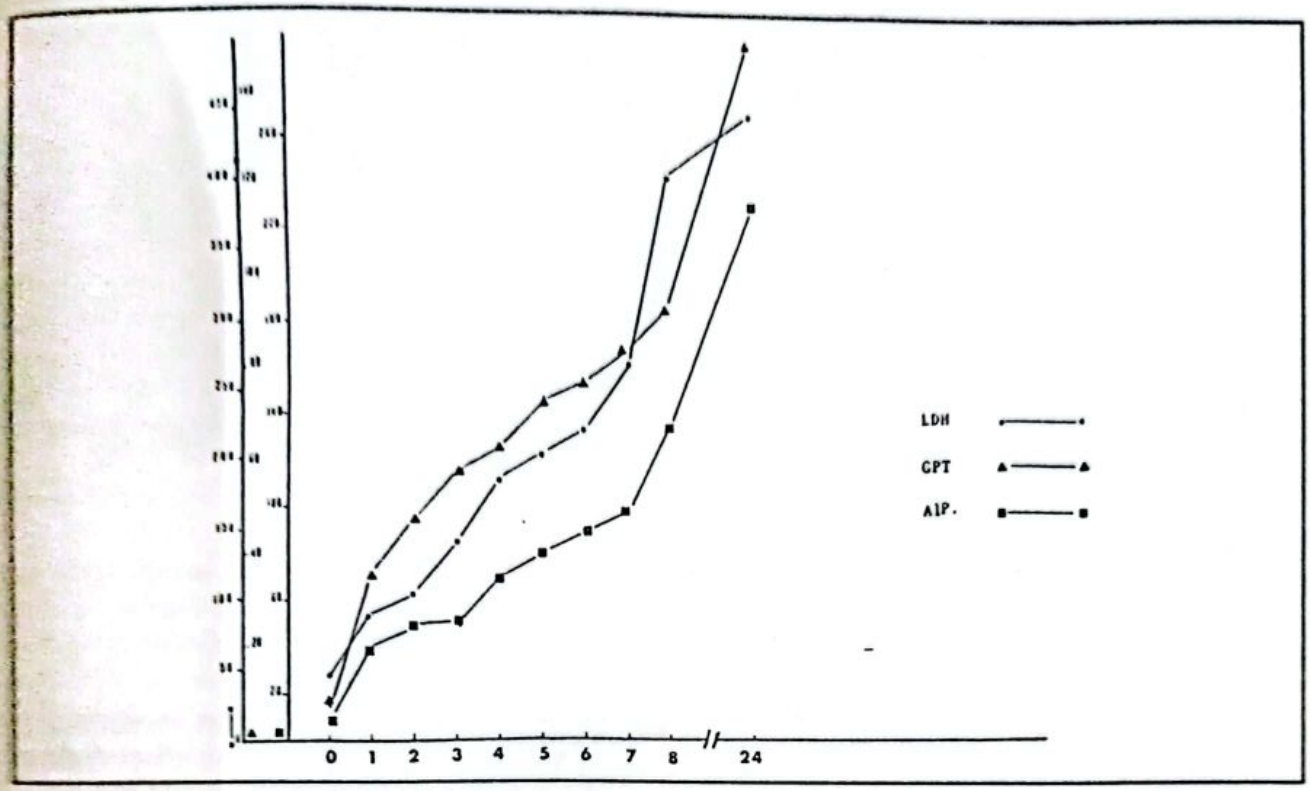


Fig. 6: The changes of lactic acid dehydrogenase, GPT and alkaline phosphatasein synovial fluid of chemically induced arthritis.

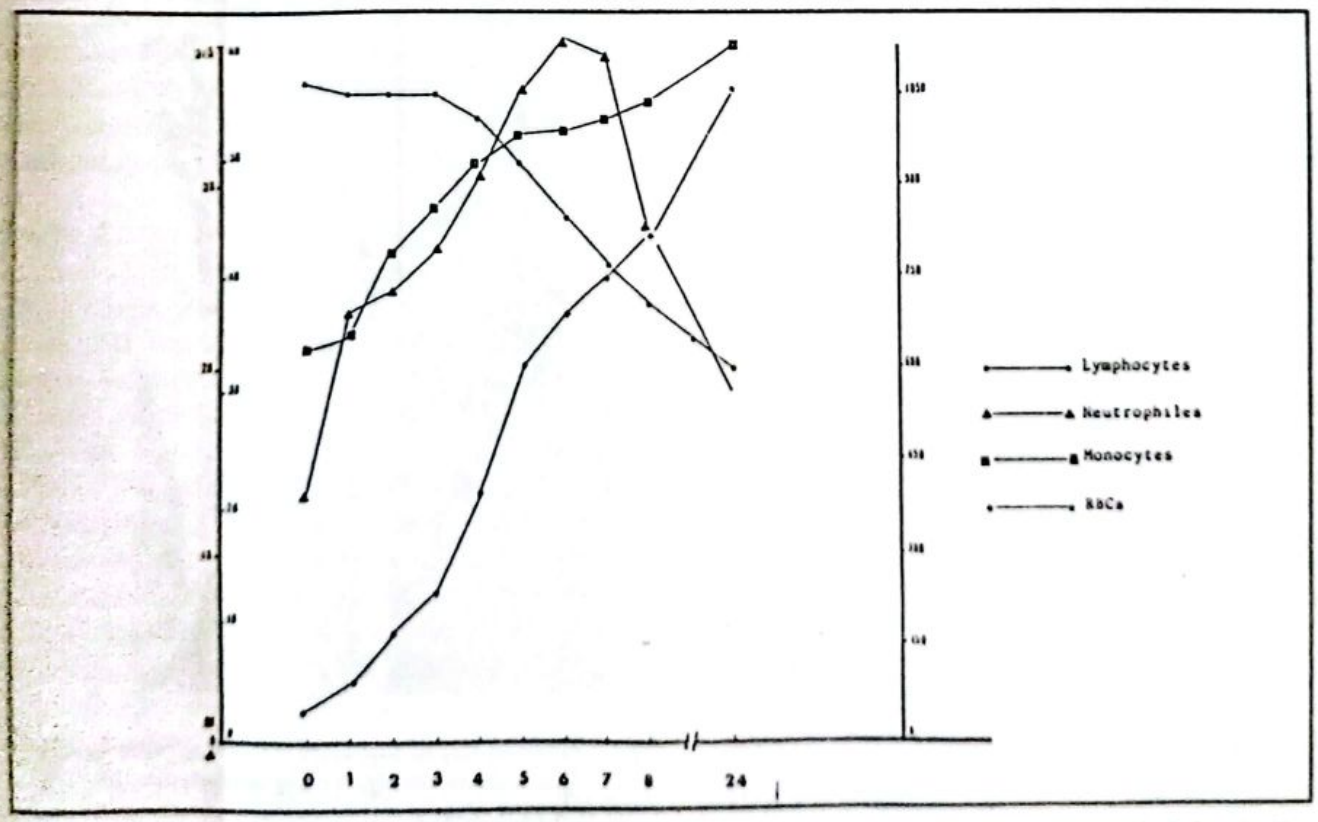


Fig. 7: The changes of hymphocytes, neutrophils, monocytes and red blood corpuscles in synovial fluid of chemically induced arthritis.

Fig. 8: Mild yellowish discoloration and loss of luster of the cartilage surface of left joint injected with amphotricine B (right joint injected wit dextrose 5% as a control).
A: Joint cavity 1 week post injection.
B: Joint cavity 3 weeks post injection. Please note the progressive changes of the synovial membrane and degenerated cartilage.

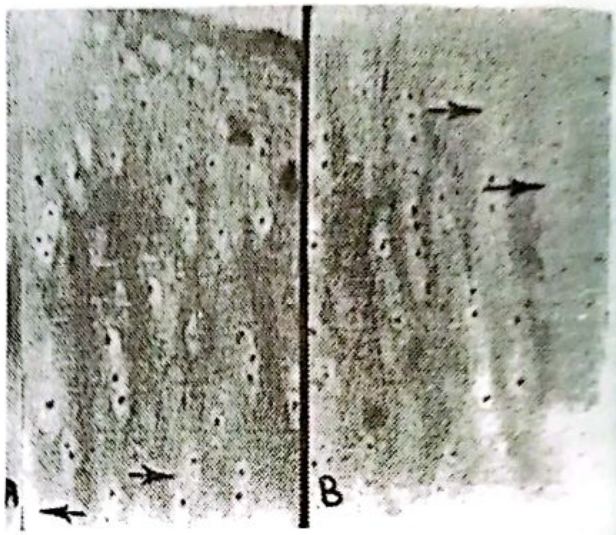


Fig.: 10: (A) Articular cartilage 1 week after induction of arthritis (B) Two weeks opst injection. Note the individual cell degeneration (arrow) and irregular staining affonity of matrix (H & E X 1 0 0) .

Fig. 9: Synovial membrane 1 week after induction of arthritis Note the increase of vascularity, partially oedematous and thickened villi (H & E X40).

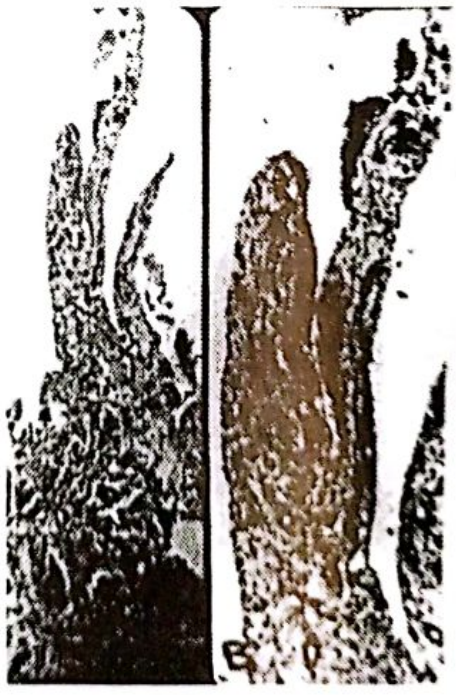


Fig.11: Synovial membrane after injection. Note interstitial oedema and thickening of the villi. (H & E A X 40 B X 100).

Synovial fluid analysis:

The changes in the viscosity, colour pH, mucinous precipitation, total protein, GOT, GPT, alkaline phosphatase, lactic acid dehydrogenease, lysozyme, hyaluronic acid, white blood cell count, red blood cell count and differential leukocytic count of the arthritic joint in chemically induced arthritis are shown in (Figures 4, 5, 6, and7). Based on this analysis, the progress in the degenerative joint disease could be classified into two main phases:

Phase I:

The first phase (first week) was characterised by highly significant increase in total protein ($P<0.01$), WBCs ($P<0.01$), RBCs and neutrophils ($P<0.01$). Highly significant decrease in mucinous precipitation ($P<0.01$), pH ($P<0.01$) and almost no changes in hyaluronic acid and viscosity could be noticed. This phase could be identified as acute inflammatory phase.

Phase II:

This phase started at the beginning of the 2nd week until the end of the experiment and could be classified into 3 stages according to the changes in the parameters examined in the synovial fluid of atthritic joint.

Stage I:

This stage started at the beginning of the 2nd week till the beginning of the 4th week. It was characterized by a significant increase in total protein ($P<0.01$), GOT ($P<0.01$), GPT ($P<0.01$), alkaline phosphatase ($P<0.01$), LDH ($P<0.01$), LDH ($P<0.01$), WBCs ($P<0.01$), RBCs and neutrophils ($P<0.01$), Hyaluronic acid and mucinous precipitation were highly significantly decreased ($P<0.01$), wherese the decrease in lysozyme was non significant. This stage may be identified as early degenerative stage.

Stage 2:

This stage started at the beginning of the 4th week until the beginning of 7th week. It was characterized by low. significant changes it he levels of the lysozyme ($P>0.05$), GOT ($P<0.05$), pH($P>0.05$), viscosity ($P>0.05$), mucinous precipitation ($P>0.05$), neutrophils ($P>0.05$), WBCs ($P>0.05$) and monocytes ($P>0.05$). Highly significant increase in total protein ($P>0.01$), GPT ($P<0.01$), alkaline phosphatase ($P<0.01$), LDH ($P<0.01$) and hyaluronic acid ($P<0.01$) and hyaluronic acid ($P<0.01$) and highly significant decrease in RBCs were also detected ($P<0.01$). The stage can be identified as a moderate stage of degenerative arthritis.

Stage 3:

This stage started at the beginning of the 7th week until the end of the experiment (24 weeks). It was characterised by highly significant decrease in viscosity ($P<0.01$), hyaluronic acid ($P<0.01$), mucinous precipition ($P<0.01$), pH ($P<0.01$), lymphocytes ($P<0.01$) and neutrophils ($P<0.01$) and neutrophils ($P<0.01$). A highly significant in LDH ($P<0.01$), Got ($P<0.01$), GPT($P<0.01$), alkaline phosphtase ($P<0.01$), WBCs ($P<0.01$), Lysozyme ($P<0.01$), menocytes ($P<0.01$) and total protein ($P<0.01$) were also noticed.

1.4.Histopathological changes:

According to the macroscopical and microscopical investigations, the changes were classified into two main phases (chronological results was illustrated in table 1).

Phase I: (Phase of acute inflammation)

This phase lasted one week. It was characterised by signs of acute synovitis in the form of redness of the joint capsule, loss of luster and consistency as well as yellowish discoloration of the articular cartilage (Fig. 8a). Microscopical investigations revealed that the synovial membrane became thicker with elongated villi. The linning synovial cells appeared cuboidal or showed stratification.

Increased vascularization with newly formed capillaries was seen in the connective tissue core. It was partaily oedematous and infiltrated with round cells (Fig. 9). The cartilage surface was rough and irregular. The lacunae appeared wide and irregularly distributed. The chondrocytes

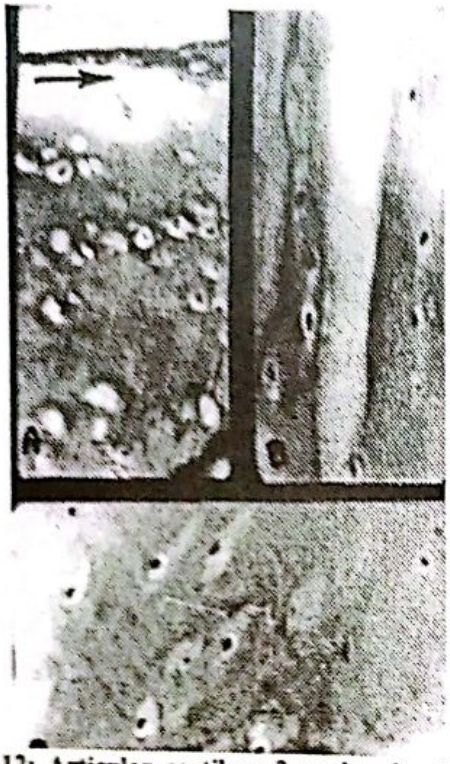


Fig.12: Articular cartilage 3 weeks after induction showing irregular rough surface and degenerated chondrocytes with multiple cyst formation (arrow) in the matrix. (H & E X100).

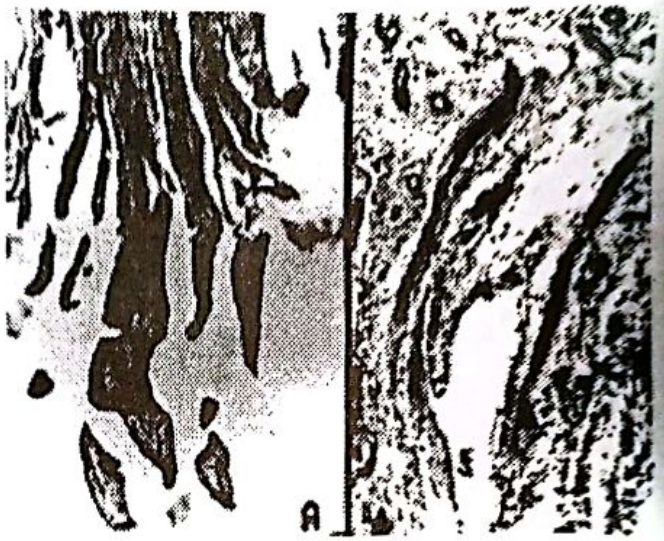


Fig.14: Synovial membrane in chemically induced arthritis after 4 weeks (A) and 6 weeks (B). Note the increase in thickness of villi and stratification of epithelial lining. H & EAX40, BX10).

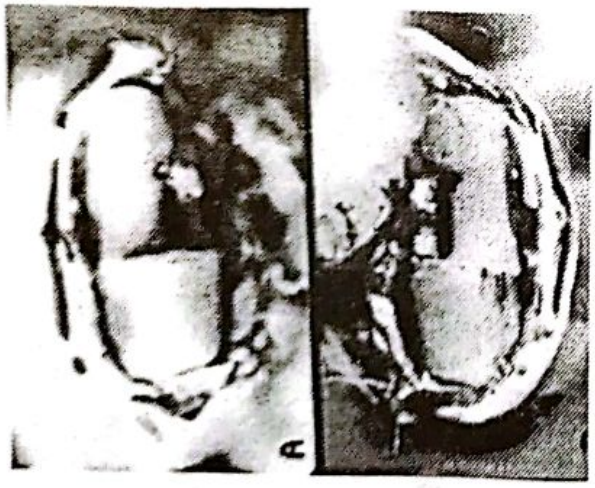


Fig.13: A: Joint cavity 5 weeks after induction.
B: Joint cavity 6 weeks after induction. Note redness of the synovial membrane, irregular surface of the cartilage and loss of luster.

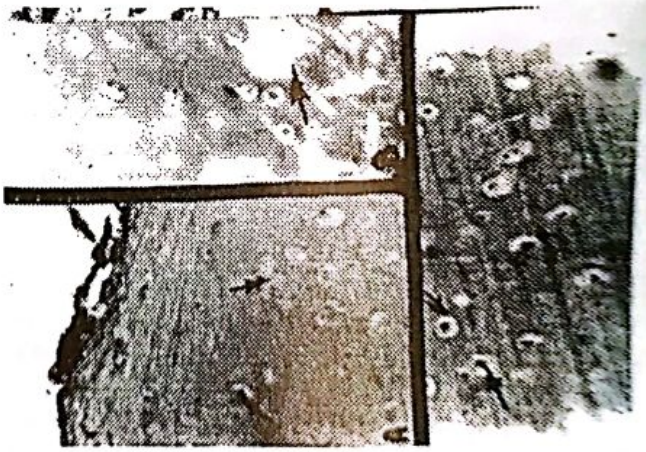


Fig.15: Articular cartilage 4 weeks after induction note roughness of the surface and cyst formation in the matrix (large arrow) with necrobioltic changes in chondrocyte (small arrow). (H & E X100).

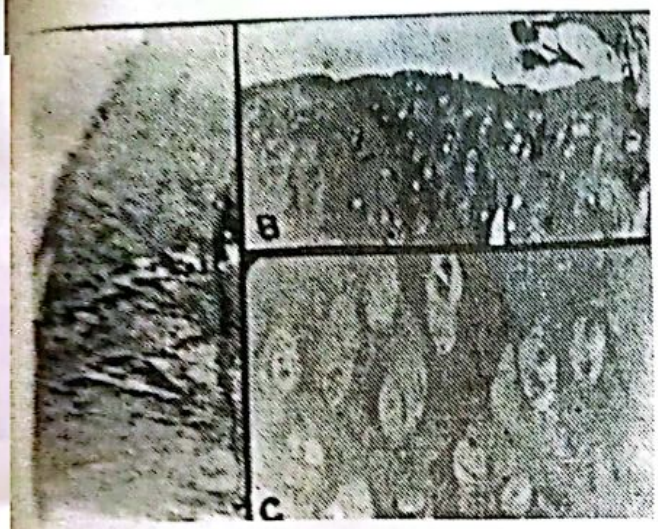


Fig. 16:Articulat cartilage 5 weeks after induction. Mote widening of the lacunae which were coafesced forming large one (arrow), fibrillation of the matrix (2 arrow) and necrobiotic changes of chondrocyte (H & E A X 40) BX100 ad CX400).

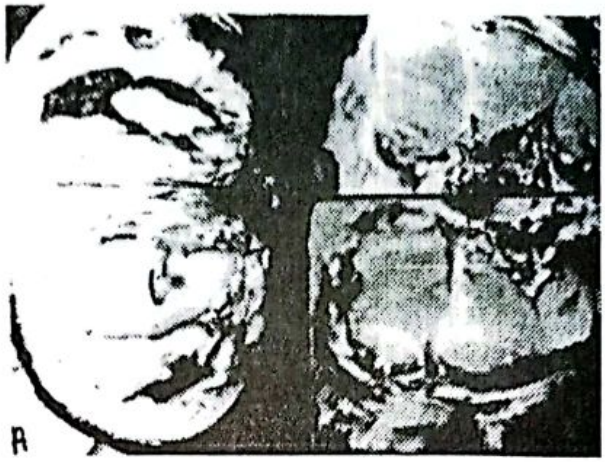


Fig. 18: (A) Joint cavity 7 weeks after induction (B & C). Joint cavity 8 weeks after induction. Note erosion in the cartilage.

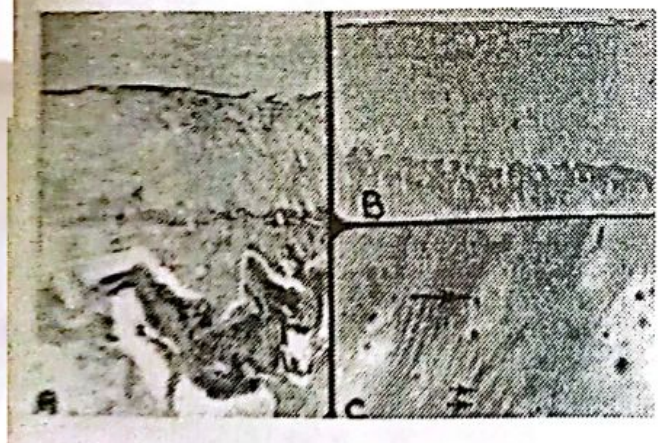


Fig. 17: Articular cartilage 6 weeks after induction. Note the progressive degeneration of chondrocytes. Pragmentation of superficial layer (arrow) and cyst formation. (H&E) AX40 B X 100 and C X 4 0 0) .

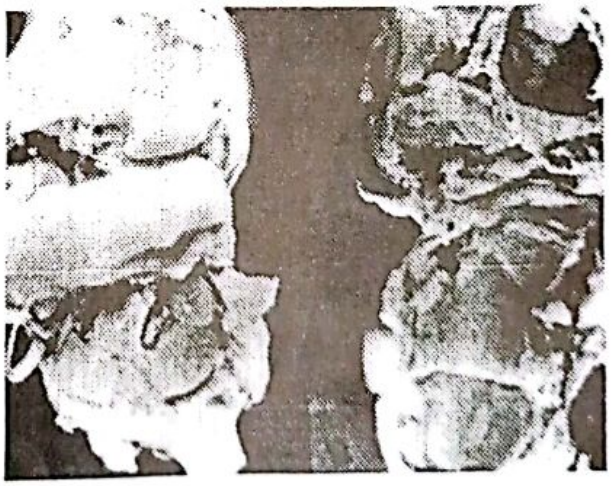


Fig. 19: Joint eavity 24 weeks after induction. Note loss of articualr cartilage and exposure of the subchondral bone.

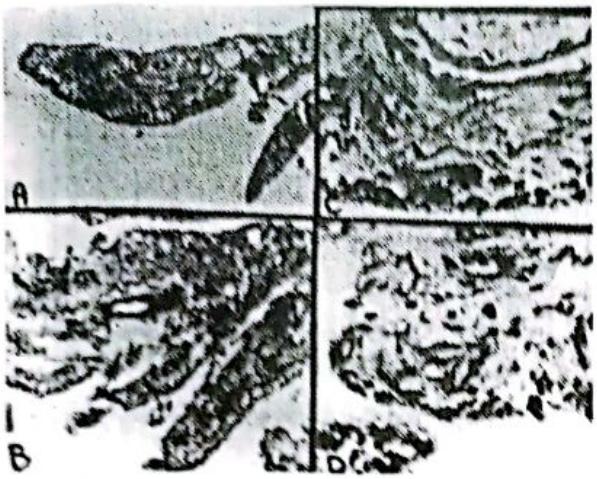


Fig. 20: Synovial membrane, chemically induced arthritis after 8 weeks of induction. Note stratification of epithelial lining, interstitial oedema (arrow) and fibrosis of the villi (H & EA &BX40 C&DX40).

Fig. 22: Articular cartilage 7 weeks after chemical induction of arthritis. Note erosion (arrow) fissuring (2 arrow), excessive dilatation of the lacunae with cyst formation and necrotic chondrocytes. (H & E A, B X 40 CX 400 and D X 100).

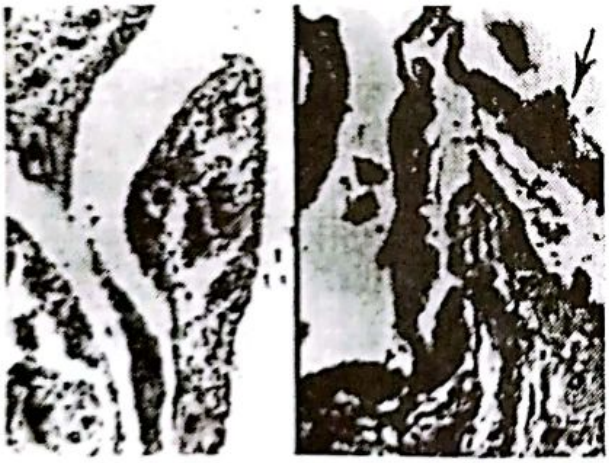


Fig. 21: Synovial membrane 24 weeks after induction. Note fibrosis of the villi, epithelial stratification and desquamation (arrow), (H&E X 100).

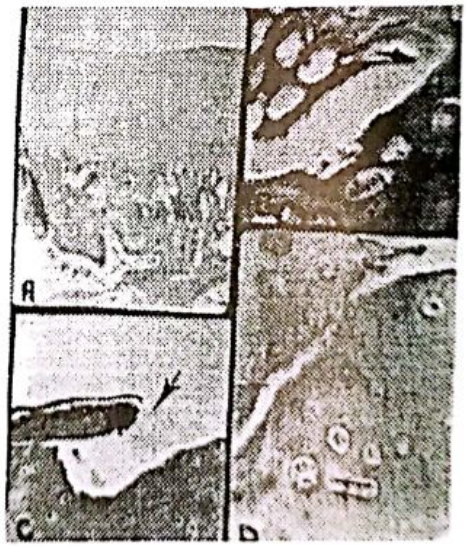


Fig. 23: Articular cartilage 8 weeks after induction. Note roughness of the cartilage surface, fissures (arrow), cyst formation, erosion (large arrow) and nest formation (2 arrows). The chondrocytes showed necrobiotic changes with excessive wide lacunae (H&E AX40 BX400 and C&DX100).

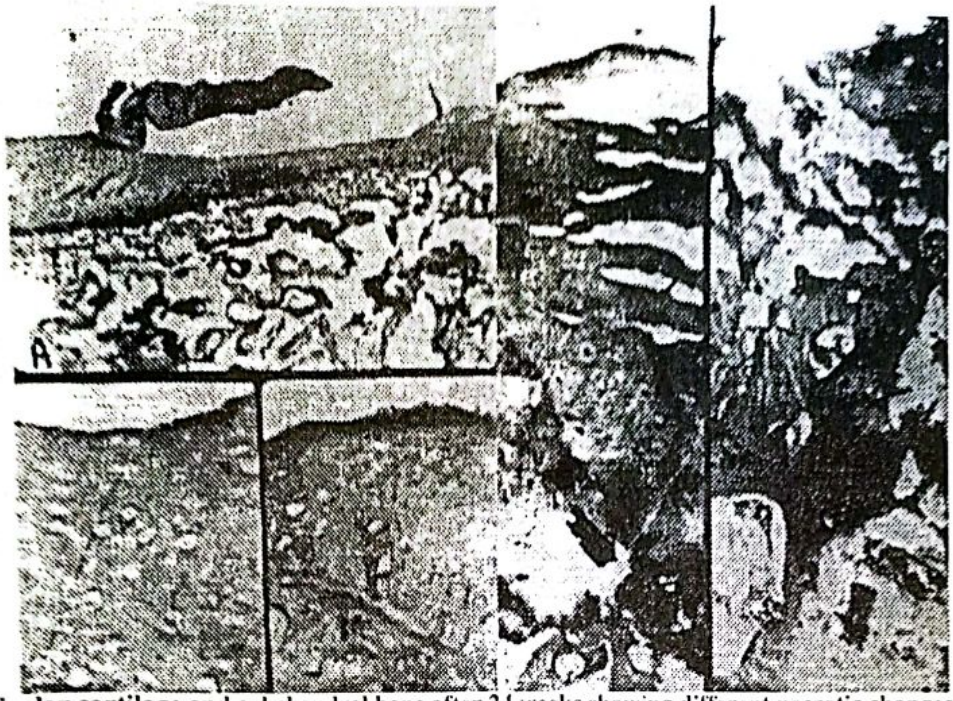


Fig. 24: Articular cartilage and subchondral bone after 24 weeks showing different necrotic changes (H & E A X 40 B, C, D, E X 100)

showed degenerative or necrobioltic changes. Fibrillation of the matrix specially the superficial layer was detected. Moreover irregular staining affinity of matrix was seen (Fig. 10).

Phase II: (Degenerative phase)

This phase divided into three stages (mild, moderate and severe) according to the severity of changes.

Stage I:

This stage started at the beginning of the 2nd wek until the end of the 3rd wek. It was characterised macroscopically by marked loss of luster, consistency and yellowish discoloration of the cartilage. It ended with early blister formation. Marked thickening and oedema of the joint capsule as well as villous hypertrophy of synovial membrane were obseved (Fig. 8B). The microscopical examination of the synovial membrane was characterised by an increase in the thickness of the villi as a result of interstitial oedema associated with round cell infiltration. Mild fibrosis was observed at the end of this stage. The epithelial lining appeared focally desquamated as early as the 2nd week and desquamation contimously increased (Fig. II). The degenerative changes of the articualr cartilage progressively increased where the cartilage surface became more irregular. The chondrocytes showed necrobiotic changes. They appeared fragmented with pyknosis, karyorrhexis and karyolysis of the nuclei. Some chondrocytes had a ghost appearance. The chondrocytes had tendency to aggregate within widening lacumae (Fig. 12).

Stage 2:

This stage started at the beginning of the 4th week until the beginning of the 7th week. The macroscopical examination of the arthritic joint was characterised by blister formation and superficial frying of cartilage (Fig. 13). Microscopical examination revealed marked fibrosis leading to thickening of the synovial membrane with formationof folds which increased in thickness as a result of fibrosis (Fig. 14). The arthritic joint showed marked degenerated chondrocytes with cyst formation. Some lacunae were widely extended and contained basophilic fluid. This was accompanied by fragmentation of the superficial layer (Fig. 15, 16, & 17).

Stage 3:

Tis stage started at the beginning of the 7th week until the end of the experiment. In this stage, there was progressive degenerative process in synovial membrane, articular cartilage and subchondral bone. The macroscopical picture of the synovial membrane was characterised by progressive aggravation of the condition with signs of chronicity at the 8th week specially villous hypertrophy and fibrosis of the capsule. There was progresive loss of cartilage and its character was manifested by superficial erosion, decrease in thickness of the cartitlage or even complete degeneration of cartilage leading to exposure of the subchondral bone. By time, eburmation (exposure of subchondral bone) was established. It was accompained with marginal lipping or osteophytosis and subchondral bone sclerosis which was clear at the 4th week (Fig. 18&19). The microscopical examination of the synovial membrane of chemically induced arthritic joint was characterised by marked fibrosis of the underlying connective tissue with intensive infiltration of round cells as well as stratification of epithelial lining and focal epithelial desquamation (figs. 20&21). The changes in the articular cartilage were aggravated where fibrillation, cyst formation with chondrocytes, necrosis and fissuring were quite clear at the 7th week (Figs. 22&23). These necrotic changes including the subchondral bony tissue were aggravated at the 24th week (Fig. 24).

DISCUSSION

Arthritis was experimentally induced in the radiocarpal joint in a series of 8 horses, 7donkeys and 7 mules by a single intra-articular injeciton of 10mg of amphotricine B. The acute inflammatory reaction developed 2-3 hours post injectio. It was characterized by lameness, swelling of the joint and hotness. This could be a result of the toxic effect of amphotricine B on the joint compartment. This observation resembles the tissue reaction in rabbit joint using streptolysine S (Weissman et . al., 1965) and in ponies using filipins (Mcllwraith et. al., 1979 A&B and Bowman et. al., 1983). However, lameness persisted longer which was possibly as a result of more delayed effect of amphotricine B than filipin (Mcllwraith et. al., 1979 A).

The signs of acute lameness during the first week as well as the synovial effusion were possibly induced by a material released from the ruptured lysosomes as the result of the destructive affect of amphotricine B (Mcllwraith et. al., 1979 A). The swelling of the joint was caused by the increased formation of synovial fluid accompanied with a reduction of resorption (Fournier et. al., 1969). Because of the persistent synovial effusion, joint capsule thickening was developed.

The clinical symptoms of srthritic joint declined gradually till it disappeared after 2 weeks except for the passive flexion which remained till the end of the experiment. However, the swelling of the joint persisted along the experimental period. The lameness could be detected in normal gait and only appeared on passive flexion of the carpal joint. This indicates that acute inflammation was converted to the degenerative form.

Radiographic evidence of degenerative joint disease (Ostephytic reaction in the form of osteoperiosteal reaction) was found in 9 out of 20 animals with amphotricine B treated joints after 7-8 weeks however all animals showed the same alteration alsong the course of the experiment. These changes were observed after 7-8 weeks, increased in severity in relation to the experimental time due to the progression of joint destruction. These findings comply with filipin iduced arthritis. The osteophytic reactions as well as the periosteal reaction in the joint were the only bony changes detected radiologically. These results run hand to hand with those reported by Mcllwraith et. al., (1979A) and Bowman et. al., (1983). The appearance of osteophytes could be interpreted as an indicator of the release of proteoglycans from the cartilage matrix as a sign of cartilage destruction. These results were correlated to those seen by Chrisman et.al., (1965), Mcllwraith et. al., (1979A), Mcllwraith, (1981) and Bowman et. al., (1983). In the mean time the histopathological examination of the arthritic joints confirmed these results.

In the present study, the synovial fluid analysis is of value to the equine practitioner as a means to determine the cause, the type (inflammatory or

non inflammatory), the duration and the stage of the disease (acute inflammatory phase or degenerative phase or destructive phase). All these information are useful in therapeutic management and its follow up as reported in our experimental work and described by Ropes (1957); Barthholomew (1968) and Van Pelt (1974). As rule, there is a close correlation between the enzyme activity in the synovial fluid especially alkaline phosphatase, lactic acid dehydrogenase, GOT,GPT and the severity of the changes in the joint disease.

Relying on the present results, the model of chemically induced arthritis could be classified into 2 main phases.

The first phase is characterised by highly significant increase in the total protein, GOT and lysozyme which act as indicator for the first phase. An increase in neutrophiles and mucin precipitation were also seen. This first phase was considered the acute inflammatory phase. These findings agree with that reported by Matthew et. al., (1962); Hunt (1965); Anon (1966) and Mellwraith et. al., (1979 A&B). The high increase in lysozyme could be considered as an indication of the onset of degnerative joint disease (Clyne, 1987). The increase in total content i the synovial fluid is attributed to an increase in capillary permeability which permits the higher molecular weight protein fraction to enter the joint (Curtis, 1964).

The second phase was characterised, in addition to the progressive changes of the synovial fluid, by a progressive sharp decrease in hyaluronic acid levels. According to the levels of hyaluronic acid during this phase. It was divided into 3 stages. It manifested itself by a high decrease in hyaluronic acid level for 2 weeks (first stage) followed by stability of hyaluronic acid level for about 3 weeks (second stage) then by another sharp progressive decrease in its level till the end of the experiment (third stage).

This decrease in the hyaluronic acid levels could be correlated to the destruction of the cartilage matirx as well as proteoglycane as mentioned by Thompson and Robinson (1981) and Cylne (1987). So, the previously mentioned findings could be considered as an evidence of functional abnormality of the joint as reported by (Persson, 1971). Also as a result of reduction in quantity of hyaluronic acid, permanent passage and retention of proteins in synovial fluid could be occured as reported by Cohen (1967) and Schubert et. al., (1968).

The first stage started at the beginning of the 2nd week until the end of the 3rd week. It was characterised by sharp significant decrease in hyaluronic acid associated with reduction in the viscosity and mucin precipitation and a high increase in protein concentration, monocytes and neutrophil count as well as GOT, alkaline phosphatase and LDH levels. It can be considered as mild stage of degenerative arthritis.

The second stage of the second phase of the chemically induced degenerative arthritis started from week of the study until the beginning of the seventh week. It was characterised by mild progressive chagnes in all synovial parameter (increase GOT, GPT; ALP; LDH; Lysozyme and WBCs, monocyte and neutrophil count with decrease in viscosity and mucinous precipitation) indicating progressive destruction of the articular cartilage. It can be considered as a moderate stage of degenerative arthritis.

The third stage of the second phase started at the beginning of the seventh week till the end of the study (24 weeks). It was characterised at first by another sharp reduction in hyaluronic acid levels and neutrophil count and a sharp increase i alkaline phosphatase, GOT and LDH levels. In addition to sharp increase in monocyte. These changes in synovial parameters can be interpreted as a result of the synovial membrane inflammation as well as massive matrix destruction which was confirmed by the pathological examination which indicate exfoliation of synovial cells. This was partially responsible for hyaluronic acid formation in addtion to escape of the destructive enzyme from degenerated chondrocytes (Mellwraith, 1982 and Clyne, 1987).

According to the macroscopical and microscopical investigations, the first acute inflammatory phase was characterised by

discoloration and softening of the cartilage. These changes could be a result of an increase in water content caused by increased water binding to collagen as reported by Mankin (1974) or damaged collagen failing to oppose the swelling pressure of the proteoglycane as mentioned by Mankin and Thrasher (1975). The huperaemia of the synovial membrane with its thickening and the increased synovial villi formation were observed macroscopically and microscopically. These changes are the result of an increased vascularization and water content as reported by McIlwraith et. al., (1979 A&B). The early microscopical alteration was confined to synovial membrane and characterized by vascular hyperaemia and edema which lead to increased thickeness of synovial villi moreover the cartilage matrix was the predelection site of chagnes and characterized by fibrillation (King, 1935 and Persons, 1971). The changes of synovial membrane were confined to either epithelial desquamation or stratification associated with fibrosis of the matrix. The decrease in hyaluronic acid in the synovial fluid as stated in the present investigation may in turn affect the matrix and lead to its fibrillation (Collins, 1949; Freeman, 1972; McIlwraith et. al., 1981 and Clyne, 1987). The presence of the round cell infiltration in the synovial membrane in addition to matrix fibrillation and chondrocyte degeneration were indicative for an acute reaction which could to amphotricin B as recorded by Lioyed-Roberts (1953) and McIlwraith et. al., (1979A&B). The clinical, radiological, biochemical analysis of the synovia and the histopathological findings cleared that the changes of synovial membrane could be regarded as early chages of degenerative joint disease before any other changes occured in articular cartilage. This opinion is supported by Raker et. al., (1966); Pond (1971); Mankin (1974) Howell et. al., (1976) and Auer (1980 A & B). The severe cellular defects of the induced arthritic joint (marked loss of lusterness with yellowish discoloration of the cartilage, blister formation and villous hypertrophy of the synovial membrane were seen macroscopically. In addition the retrogressive changes of chondrocytes and irregular surface of the cartilage which appeared microscopically were considered a feature of osteoarthritic changes (Meathin and Collins, 1962; Mankin, 1974 and Sokoloff, 1979). These changes are considered the first stage of the degenerative phase of arthritis that started at the beginning of the 2nd week and lasted to the beginning of the 4th week. The aggravated changes in the articualr cartilage as fibrillation, erosion, cyst formation with chondrocyte necrosis and fissuring were clearly seen after the 7th week. The necrobiotic chagnes of the cartilage surface was increased progressevely including the subchondral bone till the end of experiment (24 weeks).

The previously mentioned histopathological changes could be due to the loss of proteoglycanes possibly due to the great extractibility in injured tissue. Increased chondrocyte activity takes place to replenish the proteoglycans but the newly synthesized proteoglycans are of lower molecular weight and have an altered glycosaminoglycan composition as reported by McDevitt et. al., (1974) and do not readily aggregate. As a result of these changes there is a loss of elasticity and surface integrity of the crtilage resulting in increased friction and causing blistering and finally ulceration in advanced stages. The further changes was occurred due to lysozyme release from chondrocytes resulting in matrix destruction in combination with degrading enzymes from the synovial fluid that enter the matrix and lead to blistering and ulceration which causes further degradation as recorded by Thompson and Robinson (1981).

Dependding on the previous results, it could be concluded that amphotricine B has an early toxic effect on cartilage matrix leading to degraation of glucosaminoglycan (McIlwraith, 1981; Bowman et. al., 1983 and Clyne, 1987), as well as synovial membrane leading to synovitis and capsulitis. The latter charge was associated with release of lysozymes that have destructive effect on transional zone of articualar cartilage (proteoglycane and glycosaminoglycane) (Chrisman et. al., 1965). In addition, it has direct toxic effect on chondrocyte resulting in ostroarthritic changes in the injected joint (First week after induction).

As a result of prolonged destructive capsulitis, suppressio of chondrocyte methabolism and

synthesis of proteoglycane beside its possible effect on prostaglandin production, all these factors could lead to destructive effect on chondrocytes (Torback and prieur, 1979).

The previously mentioned changes in addtion to reduction of hyaluronic acid content could result in softening and loss of elasticity. The contious alteration of cartilage matabolism will lead to fibrillation of tangential zone, blister formation, frying and thinning of cartilage (Clyne, 1987) (2-3 weeks after induction). With progress of the condition, there was fissuring in radial zone with cyst formation (Clyne, 1987) (4-6 weeks after induction). With progress of the disease, osteophyte formation was present which could be detected radiologically after 7-8 weeks (Mcllwraith, 1981) as well as increase in LDH, AP and neutrophil and reduction of hyaluronic acid in synovial analysis. This is inaddition to superficial erosion, deep erosion, ulceration, cyst formation and fissuring which ended with eburnation (Mcllwraith, 1981 and Stachek, 1987).

It is concluded that the degenerative joint disease is a progressive disease that ends with complete destructuion of the joint and synovial analysis could monitore the stages of the degenerative joint disease and acs as a tool of diagnosis and prognosis early diagnosis and treatment with proper medicament prevent the retrogressive changes in the articular cartilage and return the joint to its normal condition.

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