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COMPARISON OF CORTICOSTEROIDS AND DIMETHYLSULFOXIDE TREATMENT IN CHEMICALLY INDUCED ARTHRITIS IN HORSES

By

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SUMMARY

Expermintally chemically induced arthritis was performed using single intraaricular injection of 10 mg amphotricine B in the therapeutic value of kenacort ® A; Hydrocortisone and Dimethylsulfoxide. Significant differences were not found between joints treated with kenacort A and Hydrocortisone acetate and both drugs had destructive effect on the joint. While in the joints treated with Dimethylsulfoxide, there was no evidence of destructive effect and the joint regained its soundness.

INTRODUCTION

Degenerative joint disease (osteoarthritis) is the result of a number of different pathological processes. The choice of treatment and its effectiveness depend on the stage of the disease. The line of treatment can be divided into three principles:

The first principle is prevention or treatment of primary causes.

The second principle is treatment of active soft tissue disease contributing to articular degeneration.

This includes: Rest, physical therapy (Milne, 1962; Radker et al., 1966 and Stashak, (1978), joint lavage (Chrisman, 1969) and hyaluronic acid for synovitis and capsulitis. Results are questionable as no healing or protecive effect is seen in degenerated cartilage (Rydell et al., 1970; Rydell and Balaz, 1971; Swanstrom, 1969; Wigren et al., 1978; Rose et al., 1979; Auer, 1980A; Auer and Fackelaman, 1981 and Ginerich et al., 1981).

The use of orgotein and nonsteroidal antiinflammatory drugs had no positive effect on degenerated cartilage, other treatments study as mucopolysaccharides (PSGA) can be used to inhibit cartilage degeneration (Kubitza, 1966, Ueno, 1973; Bach et al., 1977; Verbruggen and Veys, 1977; Tew, 1980; Hamm et al., 1984 and Ibrahim, 1987).

Hollander et al. (1951) introduced the intraarticular injection of hydrocortisone in the treatment of arthritis in man. Wheat (1955) used steroid injections into the synovial cavities of horses to treat various kinds of lameness. The practice has since gained widespread use in the treatment of traumatic and degenerative arthritis in horses (Quinlan 1959; Murdoch and will, 1962; Van Pelt, 1963; Houdschell, 1970; Van Pelt, 1971; Mackay and Milne, 1976; Vernimb et al., 1977; Bolbol and Fahmy, 1980; Owen, 1980 and Genovese, 1983). Several derivatives were commonly used for their greater pharmacological potency and quicker onset of pain relief (Murdoch and will, 1962 and Swanstrom, 1978 A & B). However, corticosteroids can have deleterious effects on cartilage (Stashak 1987).

Dimethyl sulphoxide (DMSO) was used in the horse alone or in combination with corticosteroids to reduce inflammation resulting from acut trauma (Tiegland et al., 1965 and Kollar, 1976). The main action of DMSO is considered to be the reduction of oedema (Wood and Wood, 1975). The drug has also been shown to enhance penetration of various agents through the skin. The drug also helps with the resolution of soft tissue inflammation in addition to its bacteriostatic action and produces collagen dissolution which may help in restoring pliability to fibroses (Wood and Wood, 1975). The drug has a definite antiarthritic effect that seems independent of its ability to promote the absorption of corticosteroids (Gorog and Kovacs, 1975 and Gray and Gottlieb, 1983).

The third principle is the treatment of cartilage degeneration.
This may include articular cartilage curettage (Riddle, 1970 and Ficat, 1979), osteophytic removal (Mcilwraith, 1981), radiation therapy (Clapp et al., 1963; Dixon, 1967; Adams, 1974; Gingerich et al. 1979 and Coventry and Scanlon, 1981) and in end stage disease, performing surgical arthrodesis (Mcilwraith, 1981 A).

The aim of this study is to compare the effect of intra-articular injection of corticosteroids and dimethylsulfoxide on chemically induced arthritis in horses.

MATERIAL AND METHODS

This study was carried out on 15 horses. The animals were classified into 3 groups as illusterated in the followign Table (1).

Arthritis was induced by injection of 10 mg amphotricin B (Bowman et al., 1983) into the left radiocarpal joint. The right carpal joint was injected with 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 experimental period. Radiographs were made of each carpus at the beginning of the experiment, weekly till the end of the experiment. Samples of synovial fluid were obtained aseptically by arthrocentesis (Edwards et al., 1977 and Rose et al., 1982). The synovial fluid samples were transferre to plain and EDTA capped vials for examination.

The laboratory and cytological values of the synovial fluid were evaluated at the time of arthrocentesis according to VanPelt and Connor (1963). Biochemical analysis of synovial fluid was carried through out the experimental period. Total protein (Henry, 1964), alkaline phosphatase (Sommers, 1954), glutamic oxalacetic transaminases and glutamic pyruvic transaminases (Reitman et al., 1957), lactic acid dehydrogenase (Anon, 1970) and lysozyme (Shugar, 1952) were measured. In addition, hyaluronic acid levels (Meyere et al., 1960 and Tolksdof et al., 1979) was estimated. Synovial samples were cultured and examined (Haupt, 1964).

Morphological and histopathological examinations of the articular cartilage, subchondral bone, joint capsule and synovial membrance were performed after euthanasia. Bone samples were decalcified using formic acid/HCl 10%. All sections were stained with haematoxyline and eosin according to Carlton et al. 1967.

Group No	Number of animals	Induction (amphotricin B)	Treatment 1 week post induction (Intra articular injection of)
1	5 horses	+	Kenacort A 80mg weekly for 3 successive weeks
2	5 horses	+	Hydrocortisone 125 mg weekly for 3 successive weeks
3	5 horses	+	Dimethylsulfoxid 40% weekly for 3 successive weeks.

treatment of experimentally induced arthritis was carried out by using kenacort ® A 40 mg, Hydrocortisone 125 mg and dimethylsulfoxid 40% (Welch et al., 1989) weekly for three successive weeks.

RESULTS

The weekly experimental treatment for three successive weeks 1 week after induction using:

I. Kenacort A (gorup 1)

1.1. Clinical findings:

The clinical signs were characterised by marked increase in the joint swelling as a result of the anti inflammatory effect of corticosteroid. The joint stiffness was reduced, enabling the patient to move freely. The diameter of the joint was returned into its circumference with an average of about 1.3 cm(± 0.03). The lameness was declined up till the third injection after which it disappeared. The all the signs of inflammation were gradually diminished and disappeared after the third injection

1.2. Radiological findings:

The radiological findings revealed the appearance of a very light periosteal reaction at the distal extremity of the radius (Fig. 1).

1.3. Synovial fluid analysis after treatment with kenacort A:

The changs in synovial fluid in chemically induced arthritis after one week of induction as a result of kenacort A treatment are shown in Figers (2&3). The profils of white blood cells count, red blood cell count, hyaluronic acid, mucinous precipitation, pH, alkaline phosphatase and lymphocytes are identcal and exhibited highly significant decrease in their levels ($P < 0.01$), while GOT, GPT, total protein, lysozymes and lactic acid dehydrogenase were characterised by highly significant increase ($P < 0.01$). On the other hand, neutrophils, monocytes and viscosity showed no significant changes ($P > 0.05$).

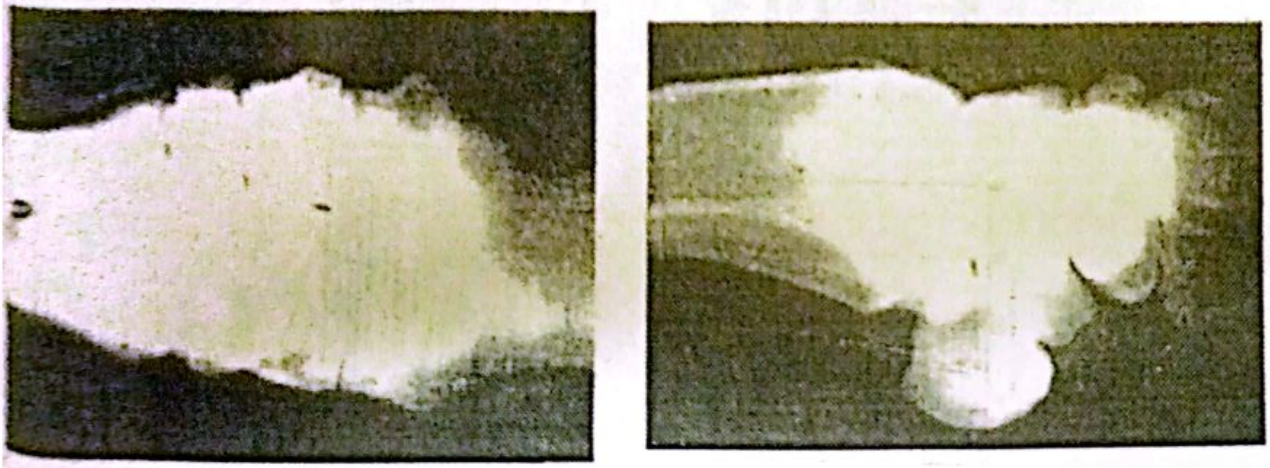

Fig. 1: Radiographs of 10 year-old horse trerated with kenacort A for three succesive weeks, showing slight periosteal reaction and minor lipping of the proximal medial aspect of radiocarpal bone. (a) Dorsal view. (b) lateral view.

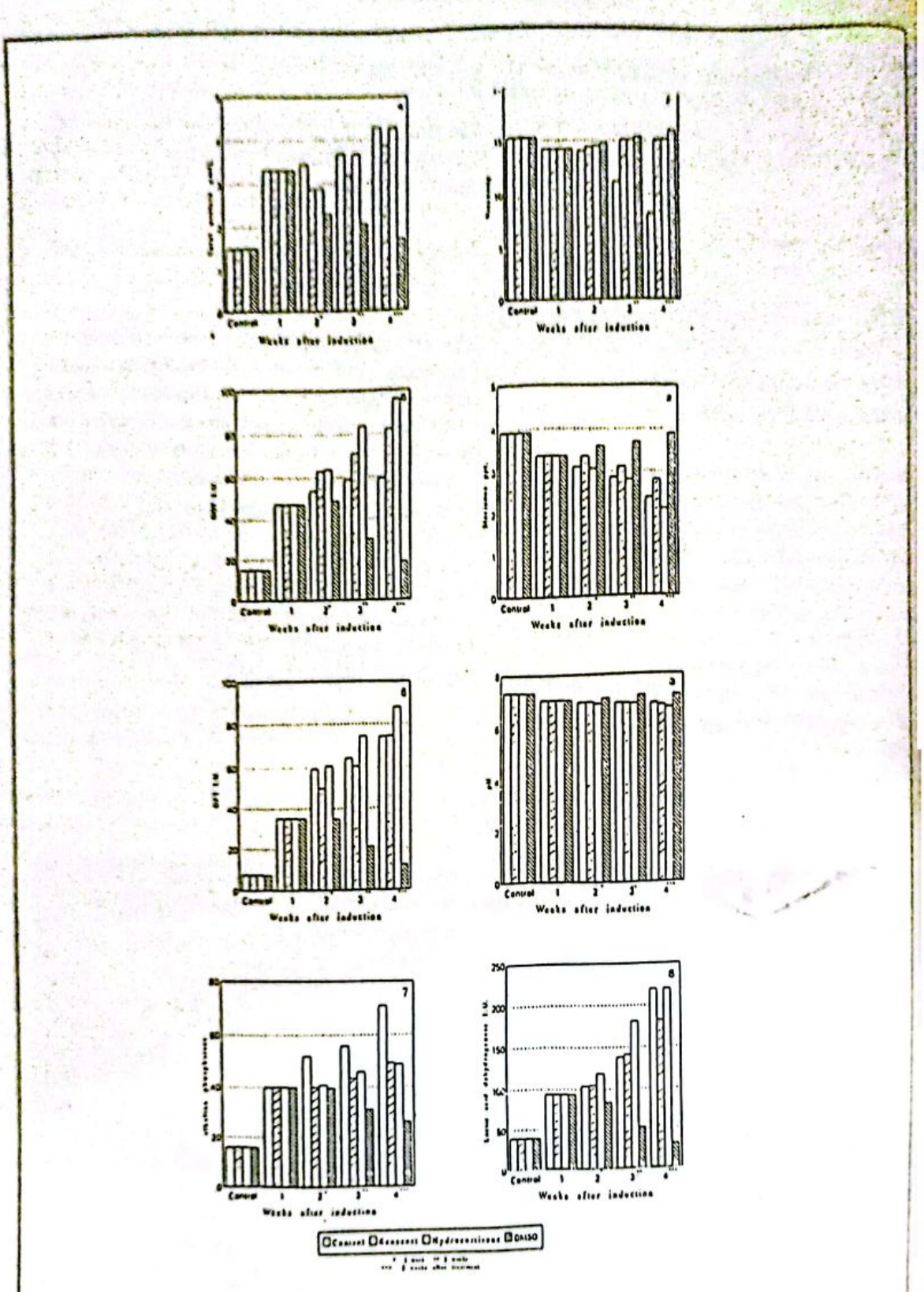

Fig. 2: The changes in synovial fluid of one week chemically induced arthritis after weekly treatment with Kenacort A, Hydrocortisone acelat and Dimethylsulfoxide 40%.
I. Viscosity 2. Mucine ppt. 3. pH. 4. Total protein 5. GOT 6. GPT 7. Alkaline phosphatase 8. Lactic acid dehydrogenase.

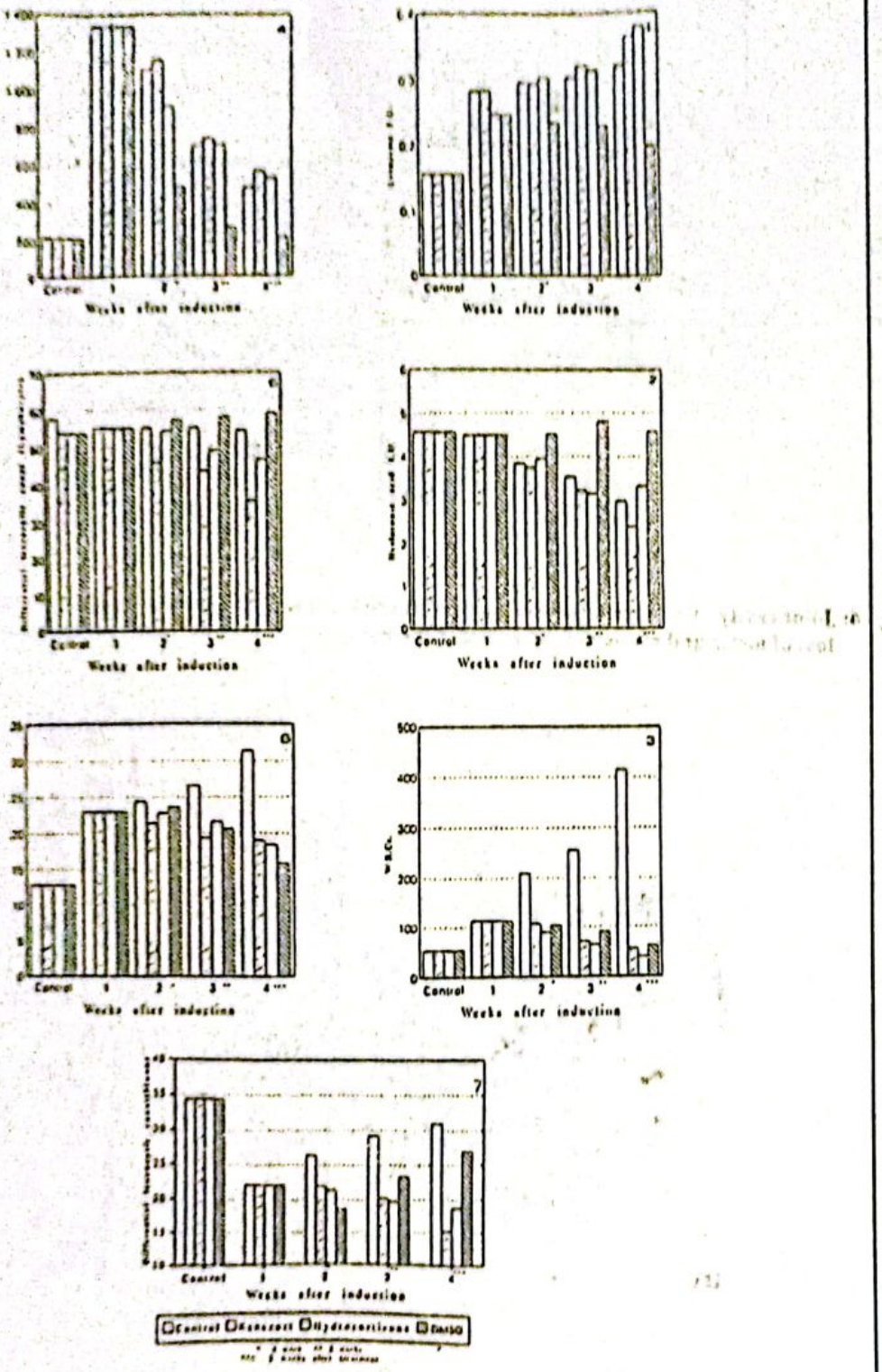

Fig. 3: The changes in synovial fluid of one week chemically induced arthritis after weekly treatment with Kenacort A, Hydrocortisone acelat and Dimethylsulfoxide 40%.
1. Lysozyme 2. Hyaluronic acid. 3. WBCs. 4. RBCs. 5. Lymphocyte 6. Neutrophile 7. Monocyte.

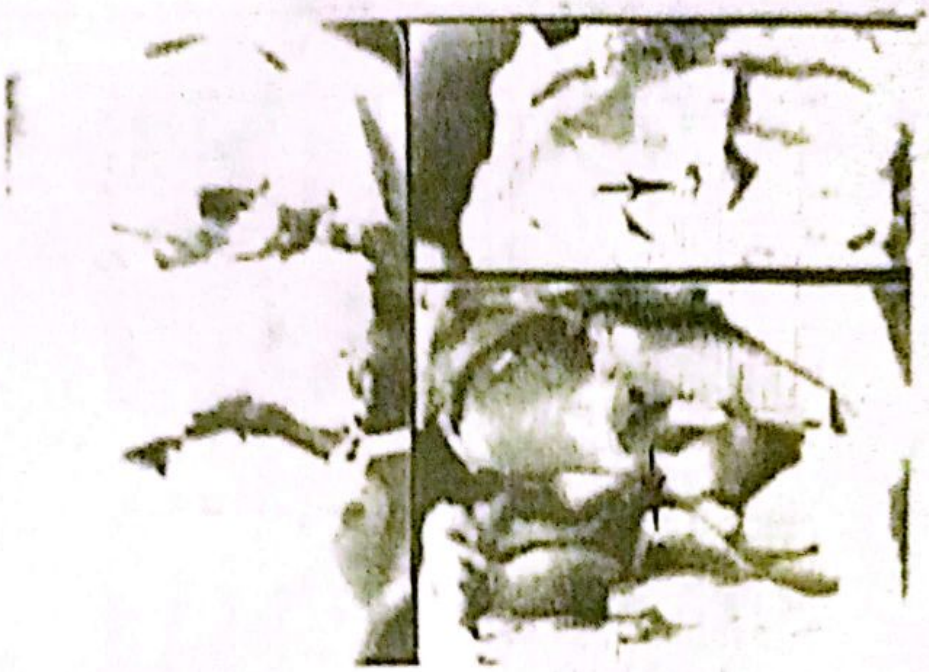

Fig. 4. Joint cavity of a horse after treatment with kenacort A for 3 successive weeks. Note yellowish discolour loss of luster and erosion of the articular surface.

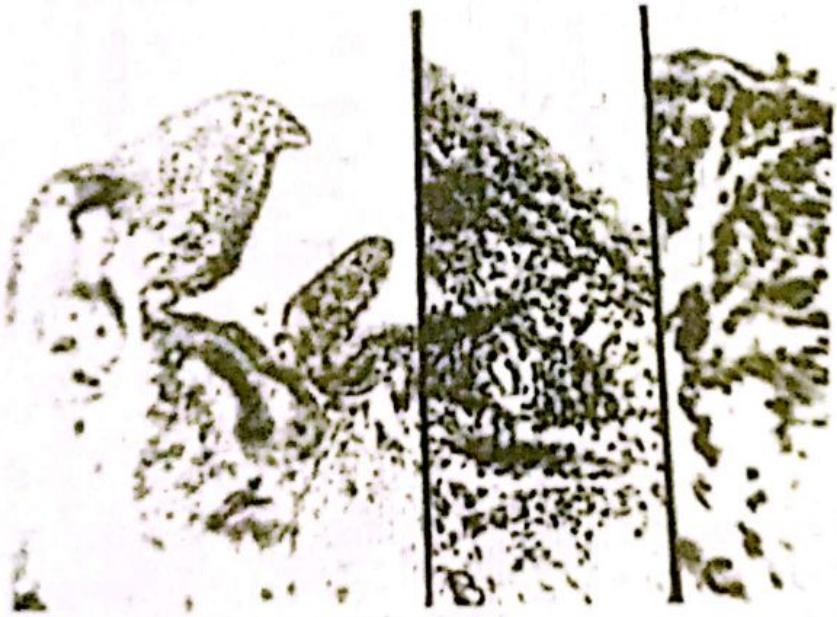

Fig. 5. Synovial membranes of an arthritic horse after treatment with kenacort A for 3 successive weeks. Note cation of epithelial lining and intensive inflammatory cell aggregation. (H&E A x 40; B x 100 and C x

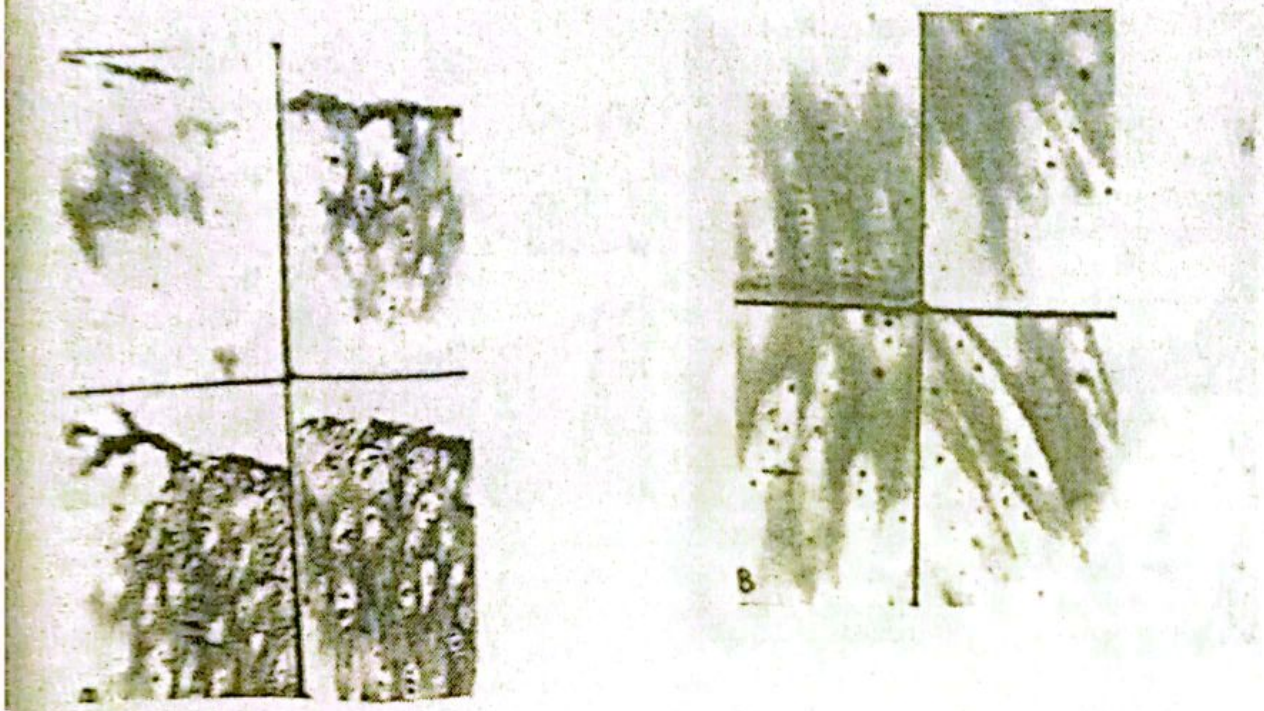

Fig. 6: Articular surface, after treatment with kenacort A (arrow and fibrillation of the matrix (2 arrow). H&E x 100).

1.4. Histopathological changes:

The macroscopical examination of the joint cavity revealed thickening of the synovial membrane with marked discoloration. The cartilage surface became rough, soft and frying with marked blister and ulcer formation (Fig.4).

The microscopical examination of the synovial membrane revealed stratification of the epithelial fibroblastic proliferation. The villi were short and nefematous (Fig. 5). The surface of the articular cartilage became irregular with multiple erosions and necrosis of its cartilaginous tissue. Different degenerative changes including myxomatous, hydropic or necrobiotic changes of chondrocytes were present. Some chondrocytes appeared ghost or shrinked with pyknotic or karyorrhetic nuclei. Fibrillation of the hyaline materix was marked with cyst formation (Fig. 6).

2. Hydrocortisone acetate (group 2):

2.1. Clinical findings:

The clinical signs were characterised by reduction of lameness and pain enabling the patient to move freely. The joint diameter wa reduced to reach 1.4 + 0.03 cm. The signs of inflammation were decreased and returned to normal condition after the third injection.

2.2. Radiological findings:

The radiological examination revealed the appearance of slight periosteal reaction on the distal extrimity of the radius and carpo-ulnar bone.

2.3. synovial fluid analysis after treatment with hydrocortisone acetate:

The changes in synovial fluid in experimentally arthritis after one week of induction as a result of hydrocortisone treatment are shown in Figs. (2&3). Highly significant decrease ($P < 0.01$) in levels of hyaluronic acid, WBCs. , RBCs. mucinous precipitation, pH alkaline phosphatase and lymphocytes was recorded. While the levels of GOT, GPT, total protein, lysozymes and lactic acid dehydrogenase were highly significantly increased. ($P < 0.01$). On the other hand, neutrophils, monocytes and viscosity showed no significant

Fig. 7: Joint cavity after treatment with hydrocortisone for 3 successive weeks. Note discolouration, superficial frying, blister formation and erosion of the articular surface.

Fig. 8: Synovial membrane of an arthritis after treatment with hydrocortisone. Note stratification of epithelial lining with intensive inflammatory cell infiltration. (H & E A x 100, B x 400).

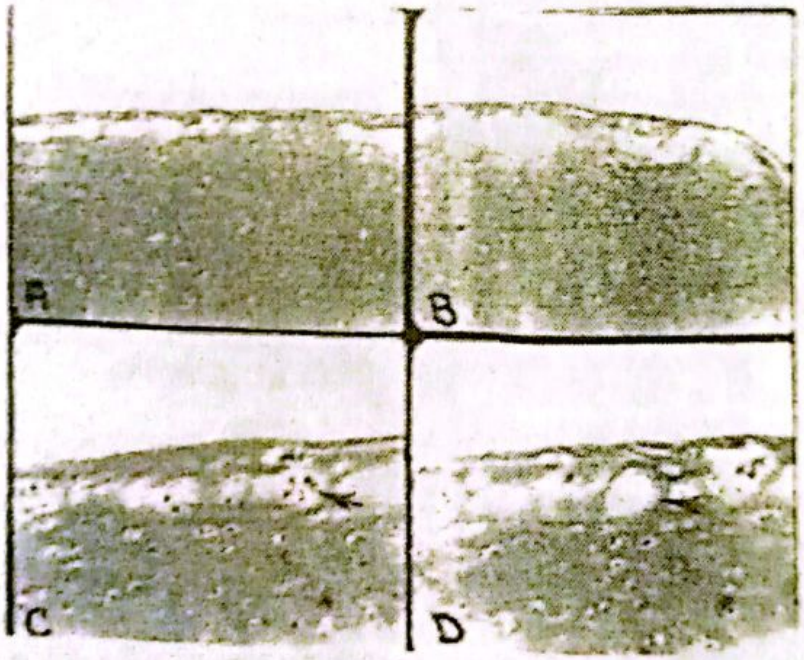

Fig. 9: Articular surface after treatment with hydrocortisone. Note degenerated chondrocytes (arrow) (H.&E. A,B 40 C; D x 100).

hanges, ($P > 0.05$).

1.4. Histopathological changes:

Macroscopical examination of the joint cavity showed thickening of the synovial membrane with marked discoloration. The cartilage surface became rough, lost its normal luster and consistency and became yellowish. Superficial frying with tissue formation and erosion were seen on the articular surface. The subchondral bone was exposed (Fig. 7). microscopical examination of the synovial membrane revealed that there were areas of stratification fo the lining epithelium and focal oedema. Intensive inflammatory cell aggregation including neutrophils, the blood vessels appeared with narrow lumen and occasionally hyalinzed. Diffuse fibroblastic proliferation in the underlying connective tissue was also detected. The synovial villi were very short and slightly oedematous (Fig. 8).

The microscopical examination of the articular cartilage revealed that the surface was irregular showing multiple erosions associated with necrosis of cartilaginous tisue. The chondrocytes showed different degenerative changes, specially those present near the surface (tangential zone) or at the junction with aubchondral bone. Some chondocytes appeared shrink with pyknotic nuclei or even had ghost like appearance. Those chondrocytes showed either myxomatous and/or hydropic degeneration. Many chondrocytes aggregated to from cell nests in which 4-5 chondrocytes were located in one lacunae. The lacunae which contained necrosed or degenerated chondrocytes appeared wide and containing eosinophilic threads. The interstitial hyaline substance showed fibrillation with multiple cyst formation (Fig. 9 & 10).

3. Dimethylsulfoxide 40% weekly (group 3):

3.1. Clinical findings:

The clinical finding were characterised by marked decrease in pain and siffiness of the joint, The animal could move freely. The diameter of the joint was reduced with an average of about 0.25±0.03 cm. The lameness was gradually improved after the first injection and completely disappeared after the 2nd and 3rd injections. The hotness of the joint was decreased and returned to its normal condition.

Fig. 10: Articular surface one week after induction of arthritis after weekly treatment with hydrocortisone acetate for three successive weeks. Note multiple erosions and necrosis of cartilagenous tissue. (H&E x 100).

Fig. 11: Joint cavity after weekly treatment with dimethylsulfoxide 40% for three successive weeks. Note normal appearance of joint cavity.

3.2. Radiological findings:

No evidence of the radiographic changes was observed.

3.3. Synovial fluid analysis:

The changes in synovial fluid in experimentally induced arthritis after one week of induction as a result of weekly dimethylsulfoxide 40% treatment are shown in Figer (2&3). There were no significant changes ($P > 0.05$) in hyaluronic acid, viscosity and lymphocytes. On the other hand, there was a highly significant increase ($P < 0.01$) in mucinous precipitation and pH; while there was a highly significant decrease ($P < 0.01$) in total protein, GOT, GPT, alkaline phosphatase, lactic acid dehydrogenease, lysozymes, WBCs. and TBCs. Significant increase ($P < 0.05$) in neutrophils also recorded.

3.4. Histopathological changes:

The macroscopical examination revealed no evidence of any changes in the synovial membrane and articular cartilage (Fig. 11).

The microscopical examination revealed that synovial membrane was lined with one layer cuboidal epithelial cells. No pathological changes could be detected (Fig. 12). While the cartilage examination revealed individual degenerated cells. The matrix was apparently normal (Fig. 13).

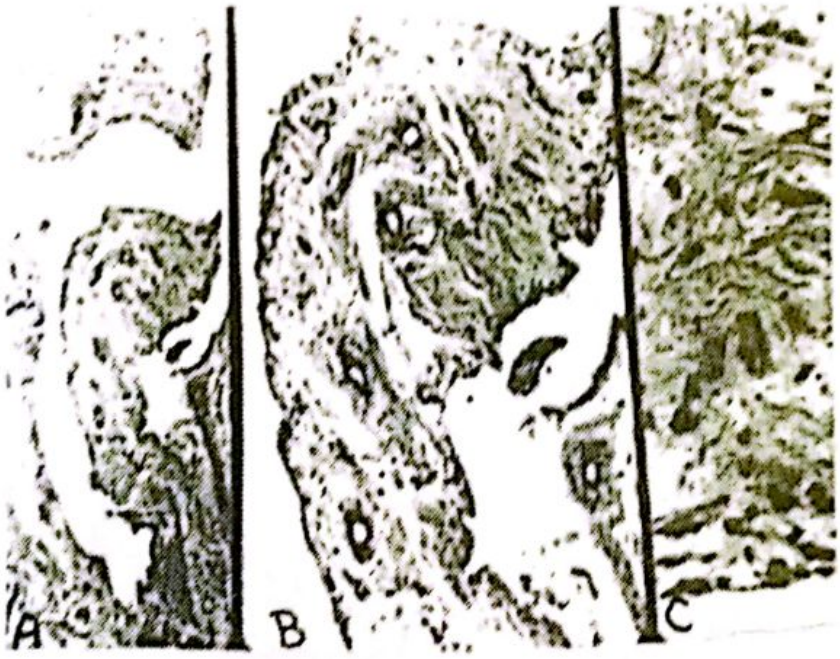

Fig. 12: Synovial membrane after weekly treatment with dimethylsulfoxide 40% for three successive weeks. Normal synovial membrane appearance. (H&E A x 40. B x 100. C x 400).

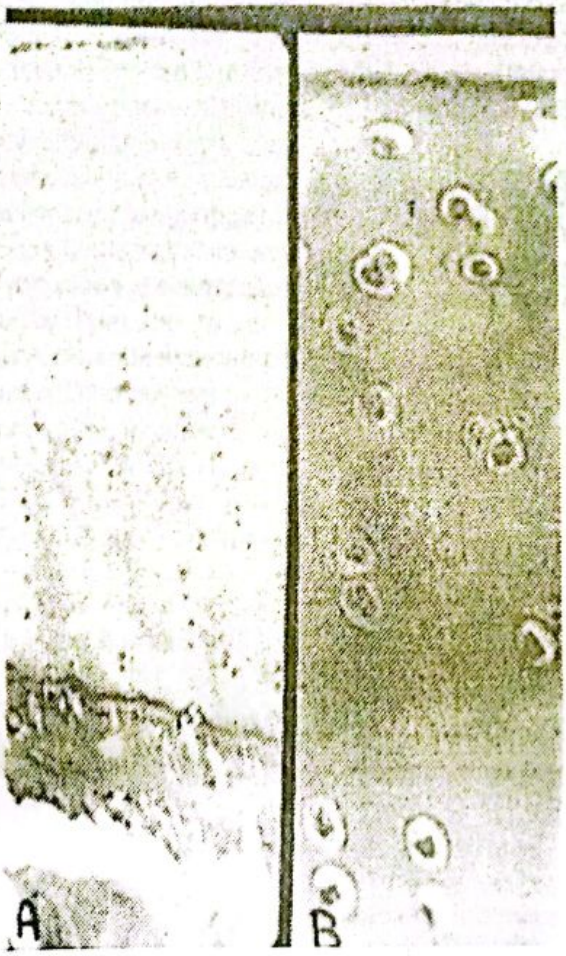

Fig. 13: Articular cartilage after weekly treatment with dimethylsulfoxide 40% for three succesive weeks Note normal appearance of articular cartilage. (H & E A x 40 B x 100).

DISCUSSION

The effect of various drugs used for treatment of arthritis after chemically (Ampho triane B induced degenerative arthritis was compared.

The effect of kenacort A® (group 1) and hydrocortisone acetate (group 2) by intra-articular injection one week after induction of arthritis (acute inflammatory phase) was evaluated over three successive weeks. It was found a marked decrease in lameness, joint stiffness and diameter while synovial fluid analysis demonstrated a reduction in mucinous precipitation, pH, lysozyme, hyaluronic acid levels, white blood corpuscle, red blood corpuscle, neutrophils and monocyte dount and an increase in total protein, GOT, GPT, alkaline phosphatase and LDH levels when compared with the right carpal joint (control negative group). This result could be due to the antiinflammatory effect of corticosteroid therapy as previously reported by Wheat (1955); Salter et al. (1967); O'Connor (1968); Van Pelt and Riley (1969B); Roach et al. (1975); Owen (1980) and Shehab (1988). Radiologically, a very light osteophytic reactions at the distal extremity of the radius was recorded. This can interpreted as a result of destructive effect of both kenacort A and hydrocortisone acetae as reported by Owen (1980) and Whehab (1988).

The histopathological examination revealed thining of cartilage, loss of elasticity, fibrillation, cyst

formation, fissuring, ulceration and degenerated chondrocyte which resulted from catabolic effect of cortisone (Owen, 1980; Vernimb et al., 1977 and Hopes, 1972).

It was obvious that cortisone enhanced degenerative disease leading to appearance of histopathological picture of joint treated with kenacort A and hydrocortisone acetate after 4 weeks to be memic with picture of joint in which chemically has been induced after 8 weeks without treatment (charcot joint) (Sulton et al. (1953); Ebadi et al. (1966); Mankin and Conger (1966 A&B); Riberio et al. 9;968); Kopta and Blosser (1969); Houdschell, (1970), Bently et al. (1975); Roach et al., (1975); Jacoby, (1976); Mackay and Milne (1976); Silberman et al., (1977); Wang et al. (1977); Ishikawal, (1978); Bolbol and Fahmy (1980); Owen, (1980); Wright and Ramos (1980); Gray and Gotllieb (1983) and silberberg et al., (1986).

On the other hand, after treatment with dimethylsulfoxid 40%, there was marked decrease in lameness, joint stifness, diameter of joint and no evidence of perosteal reaction as well as the synovial analysis indicated that all parameters were returned back to normal values as compared to negative controls. This could be interpreted as a result of antinflammatory action on synovial membrance and its effect in healing process as confirmed by histopathological findings.

After dimethylsulfoxid 40% treatment, the cartilage restored its normal structure. Dimethylsulfoxid 40% seems to be the drug of choice for treatment of synovitis and actue inflammatory phase of degenerative arthritis. This was supported by the work in chronic musculoskeletal conditions such as chronic osteoarthritis and degenerative disc diseases in humans (Demos et al., 1967; John et al, 1967; Paul, 1967 and Steinberg, 1967) which have been treated with dimethylsulfoxide in chinical trials and good results were seen in actute conditions.

It seems to be used in weekly interval with concentration 40% to give best results. DMSO which has been previously used as an analgesic drug (Alsop, 1984 and Brayton, 1986); it seems to has a great effect on prevention of cartilage degeneration. The parameters of synovial analysis which returned back to normal may be attributed to its antiinflammatory effed. It was clear that DMSO has a marked antinflammation effect since the parameters of synovial analysis responded quite well to the drug and regained normality in shorter duration when compared with corticosteroids. This antinflammatory effect may be attributed to suppression of prostaglandine and prevention of depolymerization of hyaluronic acid by oxygen derive free radical (Greenwald et al., 1976; fox, 1984 and Greenwald, 1984). In addition to reduce catabolism (Hill, 1983) and edema (Tiegland and Saurino, 167 and Ward et al., 1967). The prevention of osteophyte reaction may be attribute to its capacity in reduction of soft tissue mineralization (Demos et al., 1967; John et al., 1967; Parson et al., 1967 and Tiegland and Surino 1967).

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