

تأثير بعض الهيد وكورتيزونات والأنزيمات المذيبة للبروتينات  
فى علاج اصابات مفاصل الخيول

ع • بلبل • فوزيه فهمى

- تم حقن ثلاثون حصانا وستة وعشرون حمارا مصابة بالالتهابات المفصليّة بجرعات مختلفة من :
- ١- خلايا الهيد وكورتيزون أو هيد وكورتيزون صود يوم هيمسكسينات كل ٤ أو ٥ يوم •
  - ٢- الانزيمات المذيبة للبروتينات مثل التريسين أو الكيموتريسين أو كلاهما يوم بعد يوم •

وقد تم سحب السائل المنزلق من كل اصابة قبل وبعد العلاج لمعرفة مدى تأثير العلاج  
فى شفاء المرض •

ولقد اتضح التأثير العلاجى الاكلينيكي فى الحالات المرضية بازالة الألم وانخفاض نسبة  
الالتهاب الى جانب استعادة الحركة الطبيعية للحيوان •

ولقد وجد أن الأنزيمات المذيبة للبروتينات ذات فعالية علاجية ممتازة فى حالات وجود  
دم فى المفصل أو مرض النقرس بينما الهيد وكورتيزونات ذات تأثير عالى فى الالتهابات المفصليّة  
الأخرى •

Dept. of Surgery,  
Faculty of Vet. Med. Assiut University,  
Head of Dept. Prof. Dr. M. El-M. Monzally.

THE EFFECT OF SOME HYDROCORTISONS AND PROTEOLYTIC ENZYMES  
IN THE TREATMENT OF EQUINE JOINT DISORDERS  
( With 4 Tables )

By

ATEF E. BOLBOL and FAWZIA FAHMY<sup>‡</sup>

(Received at 1/1/1980)

SUMMARY

Thirty horses and twenty six donkeys affected with a variety of arthritides were injected varying amounts of (1) Hydrocortisone acetate or Hydrocortisone sodium hemisuccinate every 4-5 days or (2) Proteolytic enzymes, Trypsin and Chymotrypsin or both together day by day. The synovial fluid was aspirated from each condition before and after treatment to reveal the tendency for such effusion to their normal properties as well as the best drug used for such affections.

Clinical response, in all cases, was indicated by relief of pain, reduction of inflammation and the gait returned to the normal.

It was found that proteolytic enzymes have a more therapeutic effect in haemarthrosis and gout affections, while Hydrocortisones were effective in other forms of arthritides.

INTRODUCTION

Joints, tendon sheaths and bursal disorders are the most common diseases affecting equines. Aspiration of joints and synovial spaces has been performed for several years, as a diagnostic procedure (BAUER et al., 1940 and DRIKSEN, 1964) .

<sup>‡</sup> Fac. Vet. Med., Zagazig University.

Assiut Vet. Med. J. Vol. 6 No. 11&12, 1979.



In veterinary practice, diseases of the articular, ligamentous or synovial structures of limbs had commonly been subjected to blistering agents and firing. Many believe that these aids are of little value. The only advantage is that they cause the animal to be rested because of the trauma induced (WHEAT, 1955). In 1951, HOLLANDER et al. demonstrated that introduction of cortisone or hydrocortisone into the synovial cavities frequently produced marked reduction in both inflammation and pain in certain types of arthritis.

In 1952, INNERFIELD et al. recorded that trypsin therapy had a dramatic subsidence of all signs and symptoms of acute inflammation in 94.3% of the patients.

This investigation was carried to ascertain the clinical response of intra-articular, intra-bursal and intra-theal injections of both adreno-corticosteroids and some proteolytic enzymes (Trypsin and Chymotrypsin), in equines affected with a variety of inflammatory conditions.

#### MATERIAL AND METHODS

The study includes 56 patients, 30 horses and 26 donkeys, affected with various affections of joints, tendon sheaths and bursae. Aspiration of the synovial fluid from the affected synovial structures were followed by injection with one of the following drugs:

- 1- Aqueous solution of hydrocortisone sodium hemisuccinate, 50 mg/ml. (Flebocortid, Richter).
- 2- Suspension of hydrocortisone acetate, 25 mg/ml. (Hydrocortisone, Roussel).



## TREATMENT OF EQUINE ARTHRITIS

- 233 -

- 3- a-chymotrypsin, 5 mg/ml. (Alfapsin, Choay).
- 4- Aqueous solution of a mixture from a-chymotrypsin and trypsin, 2½ mg of each/ml. (Ambezim, Richter).

The first two drugs were injected every 4-5 days, while the other two were injected day by day. All drugs used were injected in combination with crystalline Penicillin at a dose varied from 500,000 - 1,000,000 I.U. according to the size of the synovial structure.

The conditions treated, number of injections, the dosage and type of treatment are shown in Table 1.

Another synovial fluid samples were collected just before the next injection of the drugs to evaluate the joint responses as determine by synovial fluid analysis.

All samples were transferred from the aspirating syring immediatly to screw-capped vials containing dried dipotassium ethylene diamine tetra-acetate (EDTA) in a concentration of 2 mg/ml of synovial fluid effusions.

Gross appearance, reaction and total volume of the synovial effusions were recorded at the time sample collection. A portion from each sample was diluted (1:10) in a standard white cell pipette with normal saline solution tinged with methyl violet to determine the total erythrocyte and leucocyte counts (modified by BOLBOL, 1975).

Samples were then centrifuged at 3000 r.p.m. for 30 minutes to obtain a supernatent fluid free from cellular materials and debris.

Mucin clot quality test was carried out after VAN PELT and CONNER (1963). The method was conducted to determine the mean



degree of polysaccharide polymerization (hyaluronic acid). The mucin clot quality was graded as follows: Normal (4); a tight ropey clump in a clear solution, Fair (3); a soft mass in a very slightly turbid solution, Poor (2) and a small friable mass in a turbid solution, Very poor (1), a few fleckes in a turbid solution.

Synovial fluid alkaline phosphatase (AP) activity was determined by the micromethod described by SOMMER (1954). The transaminases glutamic oxalacetic (GOT) and glutamic pyruvic (GPT) were estimated according to the method of REITMAN and FRANKEL (1957). Total proteins were determined by a micromethod after BIURET method (HENRY, 1964) and glucose determination was made by the method of BENEDICT (1931). Total lipids was estimated by a micromethod of ZOLLNER and KIRSCH (1962), urea, chloride, creatinine and inorganic phosphorus were determined by methods of GENTZKOW (1942), SCHALES and SCHALES (1941), FOLIN and WU (1919) and FISKE and SUBBAROW (1925), respectively.

## RESULTS

Carpal, tarsal and stifle joints are frequently the sites of traumatic injuries in horses and donkeys. Such injuries lead to impaired function, swelling, distention of joint capsule and blemishes which called "wind puffs or wind galls", "bog spavin", "carpitis or popped knee", etc... .

Synovial fluid from all joints, tendon sheaths and bursae was analysed immediately after its aspiration from the affected structures. The results of synovial fluid of carpal joints, in some cases, were recorded in Tables 2 and 3, representing the different therapeutic effect of the drugs used.



## TREATMENT OF EQUINE ARTHRITIS

- 235 -

Pre-injection analysis of synovial fluid were compared with post-injection analysis as a mean for evaluating joint responses to the intra-articular injection of Flebocortid in donkeys and Hydrocortisone in horses (Table 2), Alfapsin and Ambezim in horses (Table 3).

It was found that corticosteroids as well as proteolytic enzymes were effective in renormalization of the physical picture and biochemical constituents of the synovial effusions to a varying degree.

The effect of the drugs used in the treatment of the different cases are recorded in Table 4.

### DISCUSSION

Affection of the locomotor system constitutes the major disorders affecting equines. The acute pathological response of a joint to an aseptic injury is an acute inflammation of the synovial membrane with increases production of the synovial fluid that is deficient in hyaluronate (ROONEY, 1959).

The use of anti-inflammatory agents, corticosteroids and prteolytic enzymes, are now well established in Veterinary medicine.

The various quantities of drugs employed for intra-articular, intra-theccal and intra-bursal injections in this investigation were tolerated and provided excellent results in the majority of cases.

In general, the intra-synovial use of hydrocortisone produce a highly successful results in disorders involving synovial structures. These results were in accordance to those of



MURDOCH and WILL (1959);VAN PELT and RILEY (1968) and BOLBOL (1979). As with all drugs, certain limitations to its usefulness exist (CYRIAX and TROISER, 1953).

The anti-inflammatory action of cortecoids, following intra-articular injection are ner only to provide not only a relief from cerain existing disorders but also acts to produce a penifical changes in the synovial fluid as well as to prevent the reformation of synovial effusions. This opinion was also suggested by VAN PELT(1963) and VAN PELT & RILEY (1968).BOLBOL (1979) adviced that the treatment of synivial structures hydrecortisons exerts a strong anti-inflammatory action with reduction of the synovial effusions and its contituents rapidly returened to normal levels.

Reduction of synovial fluid volume was augmented by the use of pressure bandage. ADAMS (1974) reported that elastic material such as a rubber impregnated bandage is the most value in producing this effect;

The use of proteolytic enzymes, trypsin and chymotrypsin, gave good results in various affections of joints and tendon sheaths.

With cases of haemarthrosis and gout, such enzymes gave excellent results which attributed to their fibrinolytic and anti-coagulant action besides their anti-inflammatory effect. Moreover, the action of these enzymes on gout was not known and may be due to digestion of the crystals present by enzyme. CETRULO and NEWARK (1953) attributed the rapid healing of wounds treated with trypsin to many factors from which the digestive action of trypsin and the opening up of humoral channels of supply and excretion in the devitalized area.



## TREATMENT OF EQUINE ARTHRITIS

- 237 -

Many authors used successfully proteolytic enzymes in the treatment of several affections and diseases (INNERFIELD et al 1953; LAUFMAN and ROACH, 1953 and SHITOV and SEMENOV, 1979).

INNERFIELD et al. (1953) recorded that anti-coagulant, fibrinogenolytic and fibrinolytic effect of trypsin was apparent. It was found that trypsin activates the blood proteolytic enzyme system (LEWIS and FERGUSON, 1952). It is entirely possible that the almost immediate therapeutic response of patients with rhaumatoid arthritis to trypsin is based on lysis of intra-capillary and lymphatic thrombi in areas undergoing such types of tissue reaction (STENSON, 1951).

As a conclusion, the treatment of joints, tendon sheaths and bursae with corticosteroids and proteolytic enzymes exerts a strong anti-inflammatory action. In addition, the latter drugs had a good digestive and fibrinolytic action which was apparent in the treatment of haemarthrosis and gout.

### REFERENCES

- Adams, O. R. (1974): "Lameness in horses." 3rd Ed., Lea and Febiger, Philadelphia, U.S.A.
- Bauer, W.; Ropes, M. W. and Waine, H. (1940): "The physiology of the articular structures." *Physiol. Rev.*, 20, 272.
- Benedict, S.R. (1931): "The analysis of whole blood. II-The determination of sugar and saccharides. (Non-fermentable copper reducible substances). *J. Biol. Chem.*, 92, 141-159.
- Bolbol, A.E. (1975): "The study of synovial fluid of some domestic animals in health and disease." *Vet. M.Sc. Thesis, Fac. Vet. Med., Assiut University, Assiut.*

Assiut Vet. Med. J. Vol. 6 No. 11&12, 1979.



- Bolbol, A.E. (1979): "Trials for the treatment of traumatic olecranon bursitis (Capped elbow) in buffaloes with some corticosteroids." Assiut Vet. Med.J.,5,333-342.
- Cetrulo, G.I. and Newark, M.J. (1953): "Use of trypsin intravenously in a gunshot wound, a case report." J.A.M.A., 152, 605-606.
- Cyriax, J. and Troisar, O (1953): "Hydrocortisone and soft tissue lesions." Brit. M.J.I, 966-968.
- Driksen, Von G. (1964): "Fortschritte in der diagnostik und therapie der gelenks und schnenscheidenerkrankungen des rindes." Nord. Vet. Med., Suppl. I, 241-257.
- Fiske, C.H. and Subbarow, Y. (1952): "The colorimetric determination of phosphorus." J. Biol. Chem., 66, 375.
- Folin, O. and Wu, H. (1919): "A system of blood analysis." J. Biol. Chem, 38, 71.
- Gentzkow, C.J. (1942): "An accurate method for the determination of blood urea nitrogen by the direct nesslerization." J. Biol. Chem., 143, 531.
- Henry, R.J. (1964): "Clinical chemistry. "Harper and Row Publishers, USA.
- Hollander, J.L. Brown, E.M.; Jesser, R.A. and Brown, C.Y. (1951): "Hydrocortisone and cortisone injected into arthritic joints. Comparative effects of the use of hydrocortisone as a local anti-arthritic agent". J.A.M.A 137, 1629-1635.
- Innerfield, I.; Schwarz, A.W. and Angrist, A.A. (1952): "Intravenous trypsin: Its anticoagulant, fibrinolytic and thrombolytic effects." J. Clin. Investigation, 31, 1049-1055.
- Innerfield, I.; Angrist, A. and Schwarz, A. (1953): "Parenteral administration of trypsin. Clinical effect in 538
- Assiut Vet. Med. J. Vol. 6 No. 11&12, 1979.

## TREATMENT OF EQUINE ARTHRITIS

- 239 -

- patients." J.A.M.A., 152, 597.
- Laufmen, H. and Roach, H. D. (1953): "Intravenous trypsin in the treatment of thrombotic phenomena." A.M.A. Arch. Surg., 66, 552-561.
- Lewis, J.H. and Ferguson, J.H. (1952): "Studies on a proteolytic enzyme system of the blood. V-Activation of pro-firinolysis by trypsin." Am.J.Physiol.,170, 636-641.
- Reitman, S. and Frankel, S. (1957): "A colorimetric method for determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Clin. Path., 28, 56.
- Rooney, J. R. (1969): "Biomechanics of lameness in horses." Williams and Wilkins, Baltimore, U.S.A.
- Schales, C. and Schales, S.S. (1941): " A simple and accurate method for chlorides in biological fluids." J. Biol. Chem., 140, 879.
- Shitov, S.T. and Semenov, B.S. (1979): "Synovial arthritis in cattle." Pathogenesis,treatment and prevention." XXI world Veterinary Congress, Moscow, USSR.
- Sommers, A.J. (1954): "The determination of acid and alkaline phosphatase using P-nitro-phynyl-phosphate as a substrate." Am. J. Med. Tech., 20, 244.
- Stenson, C.A. (1951): "Studies on the mechanism of Schwarzman phenomena: Certain factors involved in the production of the local haemorrhagic necrosis." J. Exper. Med., 93, 489-504.
- Ven Pelt,R.W. (1963): "Clinical and synovial fluid response to intrasyhovial injection of 6-alpha-methyl prednisolone acetate into horses and cattle." J.A.V.M.A.,, 143, 738-748.



- Van Pelt, R.W. and Conner, G.H. (1963): "Synovial fluid from normal bovine tarsus. B. Relative viscosity and quality of mucopolysaccharides." Amer. J. Vet.Res., 24, 537-544.
- Van Pelt, R.W. and Riley, F.J. (1968): "Traumatic subcutaneous calcaneal bursitis (capped hock) in horse. J.A.V.M.A. 153, 1176-1180.
- Wheat, J.D. (1955): "The use of hydrocortisone in the treatment of the joint and tendon disorder in large animals " J.A.V.M.A., 127, 64.
- Zollner, N. and Kirsch, K. (1962): "Determination of the total lipids concentration in serum." Ges. Exp. Med., 135, 545.

TREATMENT OF EQUINE ARTHRITIS

- 241 -

Table 1

The conditions, number of injections, the dosage and type of treatment in horses and donkeys.

Condition	No. of cases	No. of injection	Treatment	Dose per injection
<u>H O R S E S</u>				
Traumatic carpalitis	2	5	Hydrocortisone	50 mg
Traumatic carpalitis	1	4	Flebocortid	50 mg
Capped knee	2	4	Hydrocortisone	75 mg
Carpal gout	1	5	Ambezim	15 mg
" "	1	3	Flebocortid	50 mg
Tendovaginitis	3	5	Hydrocortisone	125 mg
Wind galls	4	3	Flebocortid	50 mg
Bog spavin	3	4	Flebocortid	100 mg
" "	2	6	Ambezim	10 mg
" "	4	5	Alfapsin	10 mg
Carpal haemarthrosis	3	4	Alfapsin	5 mg
" "	2	5	Hydrocortisone	50 mg
Gonitis	2	7	Flebocortid	100 mg
	-----			
	30			
<u>D O N K E Y S</u>				
Traumatic carpalitis	4	4	Flebocortid	50 mg
Capped knee	3	5	Hydrocortisone	75 mg
Tendovaginitis	4	4	Hydrocortisone	125 mg
" "	2	6	Ambezim	10 mg
Bog spavin	2	4	Flebocortid	100 mg
Carpal haemarthrosis	3	5	Flebocortid	50 mg
Gonitis	5	4	Flebocortid	100 mg
Bursitis at fetlock	2	6	Alfapsin	15 mg
" "	1	3	Flebocortid	50 mg
	-----			
	26			



Table (2)

Physical and biochemical changes in synovial fluid samples following injection of hydrocortisone in carpal joints.

Test	Flebocortid		Hydrocortisone	
	Pre-injec.	Post-injec.	Pre-injec.	Post-injec.
Volume / ml	5	3	12	8
Colour	Bloody	red (sero-sanguenus)	red	reddish
Reaction (pH)	6.6	6.9	6.7	7.1
Mucin clot	1.0	2.5	2.0	3.0
RBCs (mil./cmm)	1.72	1.11	Un-counted*	2.10
WBCs (cmm)	831	650	" "	7530
Total protein g%	4.21	3.66	7.71	4.67
Glucose mg%	168.1	143.3	150.0	109.80
Total lipids mg%	96.20	52.80	181.82	120.00
Inor. phos. mg%	4.42	3.65	3.95	2.62
Creatinine mg%	3.35	1.85	2.60	2.10
Urea mg%	23.18	16.48	14.97	11.10
Chlorides mg%	443.75	413.24	399.38	340.68
A P mu/L	12.20	8.70	22.00	9.00
G O T mu/L	19.80	11.30	95.00	37.00
G P T mu/L	3.7	1.90	50.00	31.00

\* Difficult to count due to presence of mucin shreds.

Inor. phos. : Inorganic phosphorous.

A P : Alkaline phosphatase.

G O T : Glutamic oxalacetic transaminase.

G P T : Glutamic pyruvic transaminase.

TREATMENT OF EQUINE ARTHRITIS

- 243 -

Table (3)

Physical and biochemical changes in syn vial fluid samples following injection of proteolytic enzymes in carpal joints.

Test	Alfapsin		Ambezim	
	Pre-injec.	Post-injec.	Pre-injec.	Post-injec.
Volume / ml	11	5	7	4
Colour	Reddish	dark yellow	pale yellow	strew yellow
Reaction (pH)	6.8	7.3	6.3	6.9
Mucin clot	1.0	3.0	2.5	3.0
RBCs (Th ./cmm)	1,73	6.59	<b>0.540</b>	0.323
WBCs (cmm.)	1347	1018	895	630
Total protein g%	4.32	3.67	3.67	2.34
Glucose mg%	206.00	115.00	88.70	54.70
Total lipids mg%	185.40	60.61	10.80	4.40
Inor. phos. mg%	5.32	2.99	4.42	4.06
Creatinine mg%	2.60	2.10	2.35	2.00
Urea mg%	11.11	9.83	51.51	24.21
Chlorides mg%	382.73	361.25	465.94	389.65
A P mu/L	11.90	8.60	12.20	7.10
G O T mu/L	83.00	43.00	72.00	44.00
G P T mu/L	5.20	3.20	8.00	2.00

Inor. phos. : Inorganic phosphorous.

A P : Alkaline phosphatase.

G O T : Glutamic oxalacetic transaminase.

G P T : Glutamic pyruvic transaminase.



Table (4)  
Effect of the drugs used  
in the treatment of different joint cases.

Joint cases	Treatment	Results
Traumatic carpalitis	Hydrocortisone	Marked relief of local inflammation with diminution of the swellings
Capped knee	" "	
Gonitis	" "	
Carpal gout	" "	
Carpal gout	Ambezim	Relief of inflammation, crystals disappeared in synovial fluid.
Carpal hemarthrosis	Hydrocortisone	Relief of inflammation, but swelling still present, lameness occur & synovial fluid bloody.
Carpal hemarthrosis	Alfapsin	Relief of pain and inflammation, swelling subsided, synovial fluid is normal.
Bog spavin Tendovaginitis Bursitis at fetlock	All types of treatment	Pathological signs disappeared, the animal of normal gait. Hydrocortisone was more superior than others.