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LYSOZYME ACTIVITY AS A PARAMETER OF SARSITIS IN HORSES

(With One Figure)

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SUMMARY

Synovial fluid lysozyme was significantly increased P/ 0.001 in horses affected with different degrees of tarsitis compared with controls (7.17 ± 0.098 ug/ml). Lysozyme assay had proved its efficiency in early diagnosis of tarsitis in horses.

INTRODUCTION

Lysozyme, an enzyme widely distributed in many mammalian tissues and their secretions (FLEMING, 1922). The rapid lytic effect of lysozyme on Micrococcus luteus (Micrococcus lysodeikticus) provides a valuable mean for a simple assay of lysozyme activity in body fluids (OSSERMAN and LAWLOR, 1966).

There are several sources contributing to the increased synovial fluid lysozyme. Cartilage, the tissue with the highest concentration of lysozyme which is released during cartilage destruction (PRUZANSKI SAITO and OGRYZLO, 1970; TORBECK and PRIEUR, 1979). Synovial membrane lining cells and any inflammatory cells are capable of releasing lysozyme (BARLAND and NOVIKOFF, 1962; PRUZANSKI; OGRYZLO and KATZ, 1973). Leukocytes especially monocytes as well as mature neutrophils and their precursors extending back to the progranulocytes, contain significant amounts of this enzyme (BRIGGS, PERILLIE and FINGH, 1966).

MATERIAL and METHODS

A total of 62 horses were used. Twenty seven of the horses were free of any sign of hock joint affection, as clinical examination showed lack of evidence of lameness, heat, pain or swelling. Moreover there was no evidence of systemic illness. These animals were used as controls. The rest of the animals (35 horses) had different degrees of tarsitis.

Synovial fluid samples from the hock joints of the normal and affected animals (54 samples from the normal horses and 50 samples from the affected ones) were collected in clean glass tubes containing ethylenediaminetetracetate (EDTA 5 mg). The samples were centrifuged at 1500 r.p.m. for 10 minutes. The supernatants were decanted in glass vials and stored at - 70°C for lysozyme assay.

Lysozyme Assay:

The lysoplate technique (OSSERMAN and LAWLOR, 1966) was used to determine lysozyme activity. Agarose 1% was prepared in M/15 phosphate buffer pH 6.3. An acetone extract in powder form of Micrococcus lysodeicticus were suspended uniformly in a small valume of M/15 phosphate buffer solution. The suspention was then added to melted buffered agarose at a temprature of 60° - 70°C to form a final concentration of 30 mg of the organism / powder extract in 100 ml buffered agarose and poured into a petridishes to a depth of 4 m.ms. After the agarose had been solidified, 16 - 5 m.ms in diameter wells were punched through the layer with a cork porer.

Standard dilutions of purified 3X crystallized egg lysozyme in M/15 phosphate buffer which contained 1000, 500, 250, 100, 50, 25, 10, 1 ug/ml were made. The wells were filled by using capillary tubes with fixed amounts of synovias on account of using standard petri-dishes and volume of buffered agarose. The plates were then left at room temprature (26°C) for 72 hours.

The diameter of the zone of lysis of the standards was plotted against the log of the concentration of the lysozyme. From the obtained linear plot, the concentration of lysozyme in ug/ml, in the samples was determined. Statistical analysis was carried out according to SNEDICOR(1961).