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SERUM ISOAMYLASE VALUES IN NORMAL DOGS AND DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY

J.W. SIMPSON, D.L. DOXEY and R. BROWN

Department of Veterinary Medicine, University of Edinburgh, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian (Great Britain)

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## ABSTRACT

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Estimations were made of the serum isoamylase values of normal dogs and of dogs with confirmed exocrine pancreatic insufficiency. A statistically significant difference was demonstrated between the two groups in respect of the values of one of the isoamylase fractions measured. Further study has confirmed that canine salivary tissue lacks amylase activity and that the source of the isoamylase fractions was the pancreas.

This knowledge has potential value in the diagnosis of canine exocrine pancreatic insufficiency.

# INTRODUCTION

Serum amylase consists of several molecular forms, the so-called isoamylases. These act on the same substrate and produce the same products but they are produced by several different tissues (Kaneko, 1980). Previous studies suggest that the main sources of isoamylase fractions are the pancreas and intestine (Hiatt, 1959; Kramer, 1980). No amylase activity was reported in canine salivary tissue by King (1914) or Kramer (1980) but recently it has been suggested that amylase is present in salivary tissue (Cappo, 1980).

The value of total serum amylase in dogs fluctuates from day to day (Hiatt, 1959) and is considerably higher in normal dogs than in man (Kramer, 1980). In man, the urine contains large amounts of amylase and this can therefore be of diagnostic value. However, in the dog the urine contains no amylase, even when the serum value is elevated (Hiatt, 1961; Alareon-Segovia et al., 1964). This is because, in the latter species, there is total resorption of amylase in the glomerular filtrate by the renal tubules (Brobst et al., 1970).

	Norma	Normal dogs	,		Dogs with	exocrine pa	ancreat	Dogs with exocrine pancreatic insufficiency	iency
Breed	Age (yr)	Sex	Isoamylas Salivary	Isoamylase (µmol/l) alivary Pancreatic	Breed	Age (yr)	Sex	Isoamylas Salivary	Isoamylase (µmol/l) alivary Pancreatic
Collie	1.0	Ŀ	5.0	8.5	German Shepherd	1.75	FN	ي. 5	0.0
Collie	1.5	FN	6.4	14.0	German Shepherd	2.0	ſĿ,	3.4	18.1
Collie	2.0	նել	15.8	26.7	German Shepherd	2.0	նե	а <b>.</b> с	24.7
Collie	2.0	նեւ	15.4	24.6	German Shepherd	4.0	ſz.	1.1	13.9
German Shepherd	3.0	ĹЪ	5.1	17.3	German Shepherd	6.0	Σ	6.1	19.9
Greyhound	4.0	Σ	4.8	18.0	German Shepherd	8.0	Σ	6.2	24.8.
Labrador	1.5	[±.,	6.9	13.1	Labrador	2.5	Σ	1.2	14.3
Labrador	2.0	Σ	6.7	13.3	Spaniel	3.0	FN	2.8	20.7
Labrador	2.5	FN	7.3	12.3					
Labrador	3.0	Σ	16.0	32.0					
Labrador	6.5	Σ	12.5	12.5					
Retriever	3.0	Ē	5.8	18.2					
Spaniel	2.5	FN	8.9	14.1					
Mean			8.9	17.3	Mean			3.4	18.1
Standard deviation	uc		4.3	6.7	Standard deviation	u		1.7	5.5
n = 13					n # 8				

Serum isoamylase values in normal dogs and in dogs with exocrine pancreatic insufficiency

TABLE I

N = neutered

Increases in total serum amylase in dogs have been recorded in intestinal obstruction (Hiatt, 1959), uraemia and liver disease (Finco and Stevens, 1969; Brobst, 1980) and in stress or following the administration of adrenocortico-trophic hormones or morphine (Hardy and Stevens, 1975). However, acute pancreatitis is considered to be the main reason for the elevation of total serum amylase values in dogs (Brobst, 1980; Moore, 1980). The elevated values and also those of serum lipase (Hardy, 1980) have been used to assist diagnosis of acute pancreatitis.

Less work has been carried out to ascertain serum amylase values in dogs with exocrine pancreatic insufficiency. Sateri (1975) has shown that total amylase values are less in dogs with exocrine pancreatic insufficiency than in normal dogs, a finding similar to that in human patients (O'Donnell et al., 1977). In human chronic pancreatitis there may be an initial elevation and later a reduction in serum amylase values (Bouchier, 1981). Furthermore, recent work has shown a change in the isoamylase values in human patients with exocrine pancreatic insufficiency (Hasrallah and Martin, 1983).

The present study was undertaken to determine the serum isoamylase values in normal dogs and in those with exocrine pancreatic insufficiency.

## METHODS AND MATERIALS

The serum isoamylase values were determined by the Phadebas isoamylase test (Pharmacia G.B. Ltd., Hounslow, Middlesex) (Ceska et al., 1969; O'Donnell et al., 1977). In this chromogenic enzyme test, amylase hydrolises a starch polymer substrate to release a water-soluble blue dye, the concentration of which is proportional to the total amylase activity of the sample. Two separate measurements are made on each serum sample, the first to ascertain the total(T) amylase activity and the second that of the pancreatic isoamylase (R) activity. For the latter, an inhibitor with a high affinity for salivary amylase is added. The exact values for salivary and pancreatic isoamylase can be determined by comparing the R/T ratio with a curve plotted from known concentrations of standards.

Two groups of dogs were used. One group contained 13 dogs which had no history of gastrointestinal or pancreatic disease and which, following a stringent clinical examination, were regarded as normal. The other group contained 8 dogs in which exocrine pancreatic insufficiency was diagnosed. The condition was confirmed by faecal trypsin estimations (Jasper, 1954), microscopic examination of faeces for undigested food (Drummey et al., 1961), the para-aminobenzoic acid test (Batt, 1980) and the xylose absorption test (Hill et al., 1970). Further confirmation was based on response to treatment or, in some cases, established at autopsy. The breed, age and sex of each dog is set out in Table I. Blood samples were obtained by venepuncture and, after allowing the blood to clot, serum was collected and stored in clean glass containers at 0°C. Great care was taken to ensure that the serum did not become contaminated with human saliva as this contains amylase. Isoamylase estimations were carried out within 24 hours of collection. As the concentration of amylase in serum is much greater in dogs than in man, the serum samples were diluted one part serum to four parts distilled water, prior to starting the test.

In order to determine if a statistical difference existed between the isoamylase value of the normal dogs and those with exocrine pancreatic insufficiency, the paired t test or chi-squared test was used to compare the results from each group.

#### RESULTS

The serum isoamylase values for both groups are shown in Table I. For the normal dogs, the mean pancreatic isoamylase value was 17.3  $\mu$ mol/l and that for salivary isoamylase was 8.9  $\mu$ mol/l. For the exocrine pancreatic insufficiency cases, the mean values were 18.1  $\mu$ mol/l and 3.4  $\mu$ mol/l respectively for pancreatic and salivary isoamylase. Statistical analysis revealed no difference between the groups in respect of pancreatic isoamylase fractions, but a highly significant difference was found in the salivary isoamylase fractions (Table II). No difference was detected due to the age or sex of the dogs.

# TABLE II

Statistical analysis of variable parameters in the two groups of dogs

Parameter	Statistical analysis	Result	Significance
····			
Age	paired t test	1.265	NS
Sex	chi-squared	0.162	NS
Total serum amylase	paired t test	1.119	NS
Serum pancreatic isoamylase	paired t test	0.318	NS
Serum salivary (s-c) isoamylase	paired t test	3.369	**

n = 21; \*\* = P <0.01; NS = not significant; s-c = so-called

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#### DISCUSSION

Sateri (1975) demonstrated reduced values of total serum amylase in canine exocrine pancreatic insufficiency, but did not assess the isoamylase fractions. Our results show no statistical difference in total amylase or in pancreatic isoamylase values when normal dogs and those with exocrine pancreatic insufficiency were compared. There was, however, a highly significant difference in the so-called salivary isoamylase fractions (Table II).

With the exception of a recent publication (Cappo, 1980), it has been generally accepted that canine salivary tissue contains no amylase activity (King, 1914; Brobst, 1980). Our results appeared to show significant changes in salivary isoamylase using the Phadebas test and, consequently, further study was undertaken to determine the amylase activity of salivary tissue. We undertook the electrophoretic examination of canine serum and of salivary and pancreatic tissues. This revealed that there was no amylase activity in salivary tissue but that there were three distinct bands or fractions of isoamylase in both pancreatic tissue and serum. The fractions detected in the serum were similar to those found in pancreatic tissue (Brown and Simpson, unpublished observation). Accordingly, it appears that, in the dog, the Phadebas test measures different isoamylase fractions of pancreatic origin and not, as in man, amylases of both pancreatic and salivary origin. From this it may be concluded that, in exocrine pancreatic insufficiency in dogs, one of the isoamylase fractions from the pancreas is significantly reduced while the others remain unaffected. A recent study in man has also shown that isoamylase fractions from the pancreas are reduced in human exocrine pancreatic insufficiency (Hasrallah and Martin, 1983). Thus, it seems likely that, in future, single estimations of serum isoamylase fractions may be of diagnostic value.

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