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COMPARATIVE STUDY ON THE ACTIVITY OF 5'-NUCLEOTIDASE 5-NT AND GAMMA GLUTAMYL TRANSFERASE (GGT) IN SOME TISSUES OF DONKEYS, CALVES AND GOATS (THE EFFECT OF FREEZING AND THAWING)

(With One Table and 2 Figures)

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دراسة مقارنة لانشطة الانزيم ٥- نيكلتيدز والانزيم جاما جلوتاميل ترانسفيرز في انسجة الحمير والعجول والماعز وتاثير التجميد والذوبان عليها

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في هذه الدراسة تم قياس كل من الانزيم (5-NT) والانزيم (GGT) في انسجة أربعة من الحمير بعمر ٢-٦ سنوات، وأربعة من ذكور الاغنام المخصية بعمر ٦-٩ شهور، وأربعة من ذكور العجول المخصية بعمر ٦-٩شهور. في الحمير كانت الرئه أغنى مصدر لأنزيم (5-NT) بينما في الاغنام حققت الكبد التركيز الأعلى للأنزيم مقارنة بالفصائل الاخرى. عموماً قيم تركيز الأنزيم (5-NT) في أنسجة العجول والأغنام كانت أكبر منها بالنسبة للحمير. أما انزيم (GGT) فأنه يتوضح بصفة رئيسية في الكلية والبنكرياس والكبد، بالنسبة لكل الفصائل التي خضعت للدراسة. حدث نقصان بمعدل ٢٠-٢٥% في تركيز أنزيم (GGT) بعد تعرض الأنسجة للتجميد والذوبان لمدة أسبوعين بينما نقص تركيز الأنزيم بنسبة ٥٠% بعد فترة أربعة أسابيع. النقص في تركيز الانزيم 5-NT في الأنسجة بعد تعرضها للتجميد والذوبان لم يكن متسقاً خلال الأسبوعين الأولين ولكن بعد فترة أربعة أسابيع تلاحظ نقصان تركيز الأنزيم بنسبة ٥٠%.

SUMMARY

5'-nucleotidase (5'-NT) and Gamma glutamyl transferase (GGT) activity was measured in the tissues of four 2-6 years old donkeys; four 6-9 month old castrated male goats and four 6-9 month old male calves. In donkeys the lung is the richest source of the enzyme 5'-NT, whereas in goats the liver contains a higher activity of the enzyme compared with

other species. Generally the values of 5'-NT activities in tissues of calves and goats are higher than those of donkeys. GGT is mainly located in the kidneys, pancreas and liver of all the species studied. 20-25% loss in activity of GGT occurred after thawing and freezing of tissues for 2 weeks whereas more than 50% loss in activity of the enzyme occurred in four weeks period. The fall in the activity of 5'-NT in tissues was not consistent in all tissues during the first two weeks, but after four weeks period 50% loss of activity was observed.

Key wards: *Enzymes, gamma glutamyl transferase, 5'-nucleotidase*

INTRODUCTION

It has been shown that for an enzyme to be of diagnostic value it must be found within the organs and tissues of animals in different concentrations and be released into the blood plasma after cellular necrosis, increase in cell membrane permeability or proliferative changes in organelles. (Ford and Lawerence, 1965; Freedland and Kramer, (1970).

The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance. This is because the presence of these enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood (Rappaport and Wanless, 1993).

The research for serum enzymes with clinical significance remains active. The use of enzymes to monitor cell damage requires knowledge of (1) the distribution of enzymes in the organs of the various animal species (2) the variations of this distribution among cell types within a given organ and (3) the intracellular compartmentation of these enzymes (Braun *et al.*, 1983).

5'-NT is widely distributed in a number of cells and tissues. It is located in a variety of tissues, including the brain, heart, blood vessels, intestine, pancreas, and sinusoidal/canalicular membranes of the liver, where they catalyze the hydrolysis of nucleotides, generating inorganic phosphate, Friedman *et al.*, (1996). It is also considered to be related to the membrane movement of cells in the transitional epithelium, cellular motile response, transport process, cellular growth, synthesis of fibrous protein and calcification, lymphocyte activation, neurotransmission, and oxygen sensing mechanism, Vera and Jozef, (2003).

Animal 5'-NT has been classified into two major forms according to its biochemical properties: a membrane-bound form and a soluble form, Zimmermann (1992) Vera and Jozef, (2003).

In the horse, 5'-nucleotidase (5'-NT) activity is found mainly in homogenates of lung, kidney, small intestine, mammary gland, liver and pancreas. Lower activities are present in brain and muscle, Ford and Adam, (1981).

Serum activity concentrations ordinarily range from 17 to 183 μkat/L and correlate well with serum alkaline phosphatase concentrations, Kaplan, (1993). However, despite their wide distribution, an increased serum value is highly specific for bile stasis in the bile ducts and bile canaliculi with subsequent hepatic injury, given that the detergent action of bile acids on the canalicular basement membrane is thought to be the only mechanism by which the enzyme can gain access to the circulation, Hill and Sammons, (1967).

Gamma glutamyl transferase GGT enzymes are located in a variety of tissues, including the heart, brain, kidney, pancreas, spleen, and the biliary ductule cells of the liver, Friedman *et al.,* (1996). These enzymes catalyze both the transfer of gamma glutamyl groups from peptides to amino acids and the metabolism of glutathione conjugates.

In domestic animals, Gamma Glutamyl Transferase is mainly in (1) the kidneys, (2) the pancreas and (3) the intestine. Its liver activity is relatively high in cows, horses, sheep and goats and very low in dogs, cats and birds, Braun *et al.*, 1983. The use of plasma reference values can help to interpret the variations of serum GGT mainly in hepatobiliary diseases of cattle, sheep, goats and cholestatic disorders of dogs. Urinary GGT is a good test of kidney toxic damage, Braun *et al.*, 1983.

Ford and Evans, 1985 reported that the distribution of 5'-nucleotidase (5'-NT) activity in the tissues of the sheep differs from that of gamma glutamyl transferase (GGT). Nevertheless, both enzymes are released into the plasma of sheep which have been infected with Fasciola hepatica or in which the bile duct has been ligated.

The purpose of the present investigation was to study the tissue distribution of 5'-NT and GGT in different animal tissues in order to provide basic information which would add to knowledge of their academic value. Moreover, the effect of freezing and thawing at 4 °C were also studied.

MATERIALS and METHODS

Tissues were collected from four, 2 to 6 year old donkeys, which were destroyed by shooting because they were suffering from fractures or incurable lameness. All are males and were in good condition and their appetites were good, there was no clinical evidence of systemic disease and no gross lesions were found at necropsy. About 10g of liver, kidney, pancreas, small intestine, cardiac muscle, skeletal muscle (gracilis), brain and lung were collected within minutes of slaughter, drained free of blood and frozen at –20°C until analysed. 5'-NT and GGT activities were measured in homogenates of these tissues. 5'nucleotidase activity in tissues was measured with an assay kit (BDH Chemicals Ltd., Poole) based on the method of Persinjin Van der Silk, Timmer and Reijntjes (1969)

The activity of tissue GGT was measured with a test combination (Boehringer, Mannheim) using L-gamma- glutamyl –P- nitro aniledine as a substrate, according to the method of Szasz, (1969).

The same set of tissues were collected from each of four 6-9 month old castrated male goats and four 6-9 month old male calves at the near by Kuku abattoir.

Tissues collected from calves were treated in the same way as donkeys' tissues. 5'-NT and GGT were measured in homogenates of these tissues.

Each goat tissue was divided into two parts. The activities of 5'-NT and GGT were determined in one part immediately after collection. The other part was frozen at –20°C and the activities of 5'-NT and GGT were measured in these tissues after 2 weeks and then they were frozen again for a further 2 weeks and the activities of 5'-NT and GGT were measured again according to the methods described above.

RESULTS

Distribution of 5'-NT in tissues:

The mean tissue activities of the enzyme in donkeys, goats and calves are presented in Fig. 1. It can be seen that activity 5'-NT is found mainly in lungs, small intestine, liver and brain of all species studied. In donkeys, the activity of the enzyme in tissues is low when compared with that in goats and calves. The activity of enzyme in the lung is about twice as high as in kidney and liver. Small intestine and pancreas contain some activity. Lower activities were found in other tissues.

In goats, high 5'-NT activity was found in lungs, small intestine, liver and brain. Some activity was found in kidney, pancreas and skeletal muscle.

In calves, the activity of the enzyme is high when compared with that in donkeys and goats. Much of the activity is located in lungs, liver, small intestine and brain. There was some activity in the pancreas, kidney and skeletal muscle.

Distribution of GGT in Tissues:

The main tissue activities of the enzyme in donkeys, goats and calves are presented in Fig. 2. It can be seen that the enzyme is located mainly in the kidney, pancreas and liver of all the species studied.

In donkeys, the activity of enzyme in tissues generally seems to be low when compared with that in sheep and in calves, especially in lung and small intestine. The enzyme was found to be mainly located in kidney, liver and pancreas. Some activity was found in the lung whereas other tissues contain very low activity of GGT.

In goats, the kidney is the richest source of the enzyme. The activity of GGT in kidney is 9 times as high as in the lung, 11 times as high as in the pancreas and about 12 times as high as in the liver. The small intestine contains some activity whereas the rest of the tissues contain very low activity of the enzyme.

In calves, the kidney and pancreas exhibited very high activities of GGT compared with donkey and goats. The pancreatic activity is about 5 times as high as the hepatic activity. The lung and small intestine exhibited some activity. Other tissues show only low GGT activity.

Stability of Tissue GGT and 5-NT during Storage:

In table 1 are given the mean values and standard deviations of GGT and 5'-NT in tissues of goats after storage at –20°C.

As seen from the results, a significant difference between the values of GGT in fresh tissues and in tissues stored for 2weeks were observed in all tissues examined. Stored tissues have 20-25% less enzyme activity than fresh ones. Highly significant losses in activity occurred after thawing and freezing of tissues for a second period of 2 weeks. The activity decrease was more than 50% of the initial value.

Effect of storage on the activity of 5'-NT in tissues is also shown in table 1. It shows that the fall in activity was not consistent in all tissue samples stored over 2weeks. However significant loss of activity was observed when the tissues were thawed and frozen again for a further period of 2 weeks. It appears that the activity decrease in the four weeks period was more than 50% of the initial values.

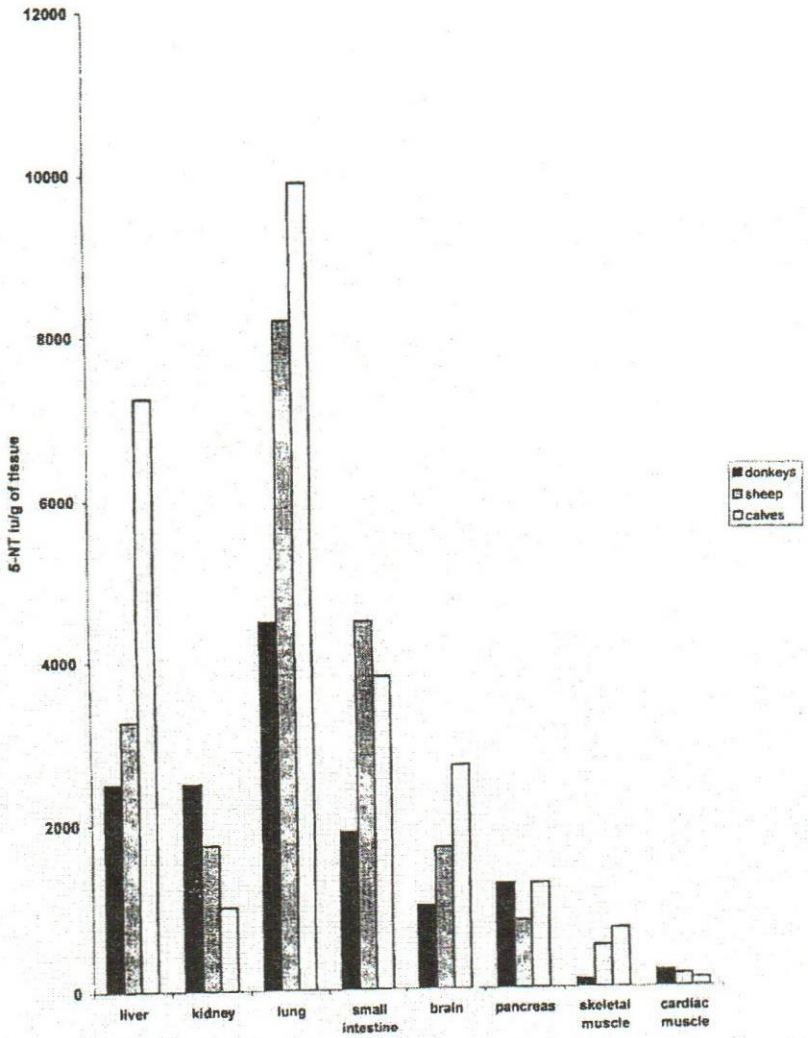

Fig. 1: Mean activities (±SEM) of 5'-NT in the tissues of Donkeys, Goats and Calves.

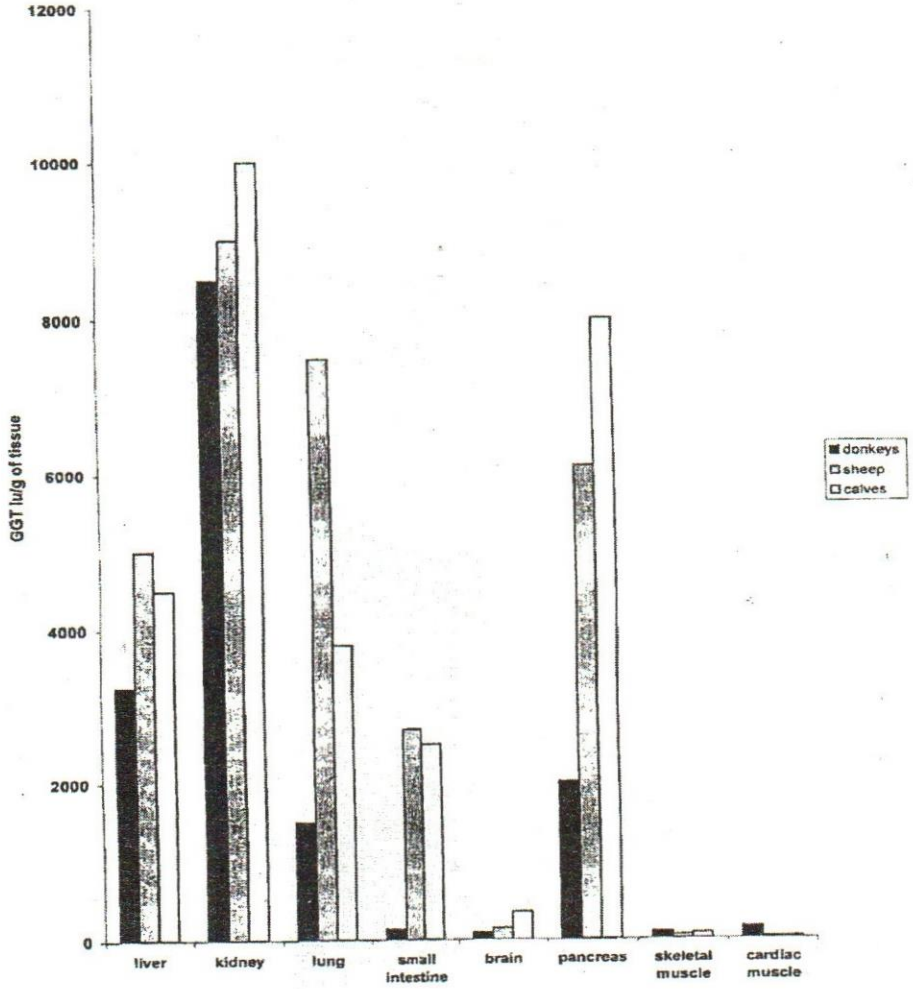

Fig. 2: Mean activities (±SEM) of GGT in the tissues of Donkeys, Goats and Calves

Table 1: The mean values and standard deviations of GGT and 5'-NT in tissues of goats after storage at –20°C

			X	S	X	S	X	S
GGT								
	Liver	4	9996	1469	7876	953	4109	962
	Kidney	4	117968	26307	91864	18260	48834	11485
	Lung	4	12928	1055	9634	361	5060	1368
	Small intestine	4	3578	1485	2075	330	1199	357
	Brain	4	476	101	354	99	165	38
	Pancreas	4	10739	1841	7393	1123	4708	1106
	Skeletal Muscle	4	28	17	0	0	0	0
5'-NT								
	Liver	4	3206	728	1983	480	1020	316
	Kidney	4	1254	237	1326	209	535	139
	Lung	4	8638	1241	5774	1449	2685	571
	Small intestine	4	4559	1169	3386	433	1600	453
	Brain	4	172	367	1152	290	491	173
	Pancreas	4	501	222	537	113	232	66
	Skeletal Muscle	4	513	130	332	50	253	76
	Cardiac Muscle	4	101	45	65	14	10	10

X S = each value is the mean of two determination on each sample

DISCUSSION

The general pattern of 5'-NT distribution and activity in the tissues of donkeys and goats are in reasonable agreement with the values obtained in horse, sheep and horse by other workers (Ford and Adam 1981; Ford and Evans 1985).

The variations in values of enzyme concentration in tissues may be due to many factors. Temperature at which the assay was carried out may affect the results. There is an approximately two fold increase in activity with an assay temperature rise from 25°C to 37°C (Freedland and Kramer, 1970). Differences in extraction procedures, age and/or sex of the animal might affect the results (Brawn *et. al.* 1983).

The results described above suggested that the activity of 5'-NT in all species studied is not specific to one tissue or organ. The lung appears to be the richest source of the enzyme in all species studied. In donkeys' the kidney, liver, small intestine and pancreas contain some activity. In goats, some activity was found in small intestine, liver and brain and in calves, the liver contains a higher activity of enzyme compared with other species. The values of 5'-NT activities in tissues of calves and goats are higher than those of donkeys. This could be due to age difference or species difference.

The results obtained suggest that the enzyme 5'-NT may be of value in assessing lung damage in these species and, considering the way in which enzyme activity varies between tissues, the enzyme may be useful in the assessment of liver damage in calves and goats.

The pattern of GGT activity in the various tissues is in agreement with that reported by Rico *et al.*, (1977); and Ford and Adam (1981) for horses by Rico *et al.*, (1977) for cattle, and by Ford and Evans (1985) for sheep.

Numerous investigators have examined the storage stability of serum or plasma enzymes. Freedland and Kramer, (1970) reported that after freezing at -18° to -20°C, the activity loss is generally about 20 to 30% over a 3week period. Johnston and Huff (1965) has reported a 30% decrease of cholinesterase activity following freezing and thawing at -4°C and at -22°C respectively, in whole blood, plasma, and serum. However, there is no previous report on the stability of 5'-NT and GGT in Tissues of animals during storage.

The present study has shown that there is a significant loss of activity in tissue GGT after freezing at -20°C for a period of 2 weeks. However the loss in activity of tissue 5'-NT during the same period is smaller. This suggests that the stability of enzymes in tissues differs from one enzyme to another. Losses of up to 50% of activity of both enzymes in tissues occurred as a result of storage for 4 weeks and repeated thawing and freezing. Disruption of cell membranes by the repeated formation of ice crystals during freezing may have caused the observed decrease in enzyme activity.

In conclusion GGT and 5'-NT were studied in some tissues of different species of animals including donkeys, goats and calves. The result was of great academic significance. The effect of freezing and thawing of 4°C was also studied and discussed.

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