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From the Department of Medicine, Royal Veterinary College, Stockholm.

TRANSAMINASE AND TRANSFERASE ACTIVITIES IN BLOOD PLASMA AND TISSUES IN DOGS

By

G. Lindblad & F. Persson

Among the transaminase enzymes that have been used for the diagnosis of tissue destruction in man and animals glutamic-oxaloacetic transaminase (G.O.T.) and glutamic-pyruvic transaminase (G.P.T.) have become increasingly valuable. Raised levels of these enzymes have been reported to occur in myocardial infarction, severe myocarditis, hepatitis, liver tumour, liver cirrhosis, muscular dystrophy, polymyositis, other extensive muscular disorders, and acute pancreatitis (*Chinsky* et al. 1956, *Wróblewski & LaDue* 1956 a and b, *Molander* 1956, *Ticktin* et al. 1957, *White & Hess* 1957, *Wróblewski* 1957, *Ersbak* 1959, *Henry* 1959). *Reichard* (1957 a, 1960) has described raised plasma-levels of ornithine-carbamyl transferase (O.C.T.) in hepatic damage in man. O.C.T. is regarded as a specific liver enzyme, for the O.C.T. activity in other organs may be considered to be of no significance. Since the variation in O.C.T. activity with time describes a typical curve in some forms of liver disease as well as of gall-bladder and duct disease (*Reichard* 1960), this enzyme should be of great diagnostic and prognostic value.

Gürtler (1960) has studied the plasma-levels of G.O.T. and G.P.T. in horses, cattle, and swine. *Wretlind* et al. (1959), and *Orstadius* et al. (1959) have made corresponding studies of tissue as well as plasma G.O.T., G.P.T., and O.C.T. in healthy swine and in swine with muscular and liver dystrophy. Determinations of G.O.T. and G.P.T. activity in dogs have been made by, for instance, *Cornelius* et al. (1959), *Lettow* (1960), *Kutas & Karsai*

(1961). Experimental work with dogs has also been carried out to investigate the G.O.T. and G.P.T. activity in various diseases (*Agress* et al. 1955, *Fleisher & Wakim* 1956, *Wakim & Fleisher* 1956, *Rudolph* et al. 1957, *Highman* et al. 1959).

No studies of the plasma-O.C.T. levels in dogs have been found in the available literature. *Reichard* (1959), however, observed that O.C.T. occurs in high concentrations in the livers of dogs, whereas the levels in other organs are very low.

In the present work we have studied the normal activity of G.O.T., G.P.T., and O.C.T. in both blood-plasma and different tissues in dogs.

MATERIAL AND METHODS

The enzyme activity of the tissues was investigated by the methods described in detail by *Wretlind* et al. and in the same laboratory. G.O.T. and G.P.T. were determined by the method of *Reitman & Frankel* (1957), and O.C.T. by *Reichard*'s (1957 a) method. The tissue specimens were taken from healthy dogs immediately after they had been killed by intravenous injection of mebumal.

Determination of the error of the method: 10 consecutive duplicate determinations of enzyme activity in various parts of the same liver gave a variation coefficient of 4.08 % for G.O.T., 13.89 % for G.P.T. and 12.01 % for O.C.T.

To obtain normal values for plasma activity for G.O.T., G.P.T., and O.C.T. in healthy dogs, two different materials, including 53 and 113 dogs, respectively, were investigated. In the latter group the mean age was higher than in the former which also included very young dogs. Enzyme activity was determined as stated above. Statistical analysis of the material was made.

RESULTS

The results of the enzyme determinations are summarized in Table 1.

To find out whether there is any difference between the examined dogs with respect to the enzyme activity in tissues, a statistical calculation was made by means of variance analysis. By regarding the values from different organs as different specimens from the same individual, the difference in enzyme activity between the organs could be demonstrated at the same time. The results are shown in Table 2.

Table 1.
Mean values for enzyme activity per g. of dry tissue. Extreme values in parentheses. Number of dogs: 4.

Tissue	G. O. T. $\times 10^4$	G. P. T. $\times 10^4$	O. C. T $\times 10^2$
Liver	12.89 (4.51—18.20)	11.09 (3.34—18.33)	1363.2 (295.8—2875.5)
Heart	23.32 (4.71—35.15)	3.36 (0.23—4.86)	1.94 (0.21—3.51)
Muscle	12.87 (3.98—20.70)	1.07 (0.27—1.66)	0.68 (0.15—1.38)
Pancreas	3.96 (1.15—7.51)	1.49 (0.36—3.13)	0.95 (0.00—5.04)
Kidney	6.34 (3.17—11.78)	2.84 (0.64—5.60)	2.77 (0.54—8.83)
Lung	1.62 (0.92—2.23)	0.10 (0.05—0.13)	1.76 (0.29—3.26)
Spleen	2.39 (0.66—3.25)	0.11 (0.06—0.19)	2.10 (0.40—4.07)
Intestine	2.91 (0.83—3.94)	0.06 (0.02—0.10)	19.24 (5.06—28.88)
Stomach	4.14 (0.94—5.53)	0.99 (0.25—2.40)	9.41 (2.15—26.36)
Lymph node	1.56 (0.42—3.01)	0.10 (0.04—0.21)	0.68 (0.00—2.13)

For calculation of the mean values of plasma-enzyme activity for the first material of 53 dogs the animals were divided into 3 age-groups as follows: Under 4 years, between 4 and 8 years, and over 4 years. No statistical differences in the values between these groups were found. When the material was grouped according to the age at sexual maturity, i. e. 6 months, the results shown in Table 3 were obtained.

After a test of the variances for the enzyme activities between young and old dogs, a statistical comparison was made between the mean values for dogs under the age of 6 months and over the age of 6 months. This comparison, carried out by classical analysis and Student's t-test, showed no difference between these values as regards G.O.T. and O.C.T. ($P > 0.05$), whereas the mean values for G.P.T. differed significantly from one another ($P < 0.001$). Therefore, and to obtain more accurate values, the mean values for a larger material of 113 dogs were calculated.

Table 2.
Variance analysis of the values for tissue-enzyme activity.

Variation	Degrees of freedom	Sum of squares	Mean squares	Ratio	F_{95}	F_{99}
G.O.T. Wet tissue						
Between dogs	3	222957	74319	4.5	2.96	4.60
Between organs (Within dogs)	9	1137608	126401	7.7	2.25	3.30
Error	27	442619	16393			
Total	39	1803184				
G.O.T. Dry tissue						
Between dogs	3	3325137	1108379	4.7	2.96	4.60
Between organs (Within dogs)	9	18060229	2006692	8.5	2.25	3.20
Error	27	6337394	234718			
Total	39	27722760				
G.P.T. Wet tissue						
Between dogs	3	20783	6928	2.0	2.96	
Between organs (Within dogs)	9	312192	34688	10.2	2.25	
Error	27	91777	3399			
Total	39	424752				
G.P.T. Dry tissue						
Between dogs	3	307604	102535	2.34	2.96	
Between organs (Within dogs)	9	4070926	452325	11.0	2.25	
Error	27	1182170	43784			
Total	39	5560700				
O.C.T. Wet tissue						
Between dogs	3	65907	21969	2.0	3.01	
Between organs (Within dogs)	8	628129	78516	7.1	2.36	
Error	24	263550	10981			
Total	35	957586				
O.C.T. Dry tissue						
Between dogs	3	1865878	621959	2.8	3.01	
Between organs (Within dogs)	8	11822193	1477774	6.5	2.36	
Error	24	6113937	226442			
Total	35	19802008				

Table 3.
Enzyme activity in blood-plasma of normal dogs under 6 months and over 6 months of age, respectively (material I). $\bar{x}$ = mean value; s. d. = standard deviation; $\varepsilon\bar{x}$ = standard error of the mean; n = number of dogs.

Enzyme	age	$\bar{x}$	s. d.	$\varepsilon\bar{x}$	n
G.O.T.	< 6 months	15.5	5.8	1.20	23
	> 6 months	18.6	7.3	1.33	30
G.P.T.	< 6 months	10.3	4.3	0.92	22
	> 6 months	20.9	6.6	1.21	30
O.C.T.	< 6 months	1.9	1.4	0.31	19
	> 6 months	2.4	2.1	0.35	27

Table 4.
Enzyme activity in blood-plasma of normal dogs (material II). $\bar{x}$ = mean value; s. d. = standard deviation; $\varepsilon\bar{x}$ = standard error of the mean; n = number of dogs.

Enzyme	$\bar{x}$	s. d.	$\varepsilon\bar{x}$	n
G.O.T.	18.1	5.9	0.56	113
G.P.T.	20.6	8.8	0.83	113
O.C.T.	2.2	1.5	0.14	113

These dogs were not grouped by age under and over 6 months, since few of them were under 6 months. The results of these calculations are set out in Table 4.

In the same way as the above comparison between young and old dogs, the mean values for the two groups were then compared.

No difference was found for G.O.T. and O.C.T. ($P > 0.05$), whereas there was a probable difference ($0.02 > P > 0.01$) for G.P.T. The statistical difference is not particularly marked, however, and hence there are reasons to suspect that it consists exactly of differences between young and old dogs. This, indeed, lends support to the results obtained in the first material, which showed that there seems to be a true difference between young and old dogs with respect to G.P.T.

Since it can thus be considered proved that the dogs in the two materials belong to the same population, we have brought

Table 5.
Enzyme activity in blood-plasma of normal dogs of various ages (materials I and II). $\bar{x}$ = mean value; s. d. = standard deviation; $\varepsilon\bar{x}$ = standard error of the mean; n = number of dogs.

Enzyme	$\bar{x}$	s. d.	$\varepsilon\bar{x}$	n
G.O.T.	17.8	6.2	0.48	166
G.P.T. (over 6 months)	20.6	8.4	0.70	143
(under 6 months)	10.3	4.3	0.92	22
O.C.T.	2.2	1.6	0.11	159

the two materials together for a calculation of the mean values. With respect to G.P.T., the dogs were divided into those under and those over 6 months of age. The results are set out in Table 5.

Provided that the material is normally distributed, the following extreme values for a 95 % range are obtained as normal values, expressed in enzyme units:
G.O.T.: 5.4—30.2
G.P.T. (dogs over 6 months): 3.8—37.4
G.P.T. (dogs under 6 months): 1.7—18.9
O.C.T.: 0—5.4

Since we can thus determine the activity of three different enzymes, and these can with good reason be suspected to be correlated, it may be of interest to know this correlation in normal dogs. To that end, the correlation coefficient between the different enzymes was calculated for material I (53 dogs) and the confidence interval was then calculated. There seems to be a close correlation between G.P.T. and O.C.T. ($P < 0.001$, i. e., significant deviation from the hypothesis of no correlation). Between G.O.T. and G.P.T. there is a moderate correlation ($0.01 > P > 0.001$), whereas there seems to be no correlation between G.O.T. and O.C.T. ($P > 0.05$).

DISCUSSION

The variance analysis (Table 2) of the values for tissue-enzyme activity shows that for G.O.T. there is some difference between the dogs up to the 99 % level ($P = 0.01$). Since there is no difference between the dogs with respect to G.P.T. and O.C.T., the number of examined dogs (4 animals) has been considered sufficient. The same analysis shows that there is a highly signi-

ficant difference between the examined organs. This is in complete accordance with what is to be expected, and a statistical analysis merely to prove this would be unnecessary.

Table 1 shows the activity of the three enzymes in various tissues. The G.O.T. activity is highest in heart muscle, skeletal muscle, and the liver. The G.P.T. level is highest in the liver, which is consistent with the observations in man but contrary to what has been found in swine in which the G.P.T. activity in the liver is reported to be no higher than in skeletal and heart muscle (*Wretlind* et al.). Hence, in dogs it seems likely that determinations of G.P.T. activity might be very useful in assessing the severity of liver damage. O.C.T. is of course still more highly specific for the liver than is G.P.T., and with regard to strength its activity in the next organ, the small intestine, is only 1.4 % of that in the liver. However, determination of O.C.T. involves time-consuming and troublesome laboratory procedures and, therefore, the finding of the high G.P.T. activity in the liver is of value. Moreover, the statistical analysis for the correlation between the plasma activity of the three enzymes reveals that there is a close correlation between G.P.T. and O.C.T. in normal plasma.

The question of how long after acute tissue damage an increased plasma activity can be expected has been made clear by earlier investigations (*Dunn* et al. 1958, *Reichard* 1959). It can be summarized that the excretion rate is highest for G.O.T., slightly lower for O.C.T. and lowest for G.P.T.

A comparison of our normal values for plasma activity (Table 5) with comparable values published by *Cornelius* et al. shows that our G.O.T. values deviate significantly ($P < 0.001$) from theirs. From 25 dogs they report the G.O.T. value of $22.7 \pm$ s. d. 5.4 Sigma-Frankel units. The statistical significance for the deviation is referable to the order of magnitude of our material.

On the other hand, our G.P.T. values show good agreement ($P > 0.05$) with theirs ($2.8 \pm$ s. d. 6.2 from 25 dogs).

Normal values for O.C.T. activity in plasma of normal dogs have not been published earlier. Our mean value of 2.2 seems to be slightly higher than the corresponding value in swine (*Wretlind* et al.) and in better agreement with the value reported in man (*Reichard* 1957 a).

REFERENCES

Agress, C. M., Jacobs, H. I., Glassner, H. F., Lederer, M. A., Clark, W. M. G., Wróblewski, F., Karmen, A. & LaDue, J. S.: Serum transaminase levels in experimental myocardial infarction. Circulation, 1955, *11*, 711—713.

Chinsky, M., Shmagranoff, G. L. & Sherry, S.: Serum transaminase activity. J. Lab. Clin. Med. 1956, *47*, 108—118.

Cornelius, Ch. E., Bishop, J., Switzer, J. & Rhode, E. A.: Serum and tissue transaminase activities in domestic animals. Cornell Vet. 1959, *49*, 116—126.

Dunn, M., Martins, J. & Reissmann, K. R.: The disappearance rate of glutamic oxalacetic transaminase from the circulation and its distribution in the body's fluid compartments and secretions. J. Lab. Clin. Med. 1958, *51*, 259—265.

Ersbak, J.: Klinisk vurdering af svingninger i transaminaseaktiviteten i serum. Nord. Med. 1959, *61*, 49—54.

Fleisher, G. A. & Wakim, K. G.: Transaminase in canine serum and cerebrospinal fluid after carbon tetrachloride poisoning and injection of transaminase concentrates. Proc. Staff. Meet. Mayo Clin. 1956, *31*, 640—648.

Gürtler, H.: Die diagnostische Bedeutung einiger Serumfermente. Zbl. Vet. Med. 1960, 7, 160—163.

Henry, L.: Serum Transaminase levels in liver disease. J. Clin. Path. 1959, *12*, 131—137.

Highman, B., Mailing, H. M. & Thompson, E. C.: Serum transaminase and alkaline phosphatase levels after large doses of norepinephrine and epinephrine in dogs. Amer. J. Physiol. 1959, *196*, 436—440.

Kutas, F. & Karsoi, F.: The diagnostic value of transaminase and cholinesterase determinations in hepatic diseases of domestic animals. Acta vet. Acad. sci. Hung. 1961, *11*, 277—288.

Lettow, E.: Transaminasen und Milchsäuredehydrogenase-Bestimmungen beim Hund. Zbl. Vet. Med. 1960, 7, 188—191.

Molander, D. W.: Serum transaminase in liver disease. Clin. Res. 1956, *4*, 63.

Orstadius, K., Wretlind, B., Lindberg, P., Nordström, G. & Lannek, N.: Plasma-transaminase and transferase activities in pigs affected with muscular and liver dystrophy. Zbl. Vet. Med. 1959, *6*, 971—980.

Reichard, H.: Determination of ornithine carbamyl transferase with microdiffusion technique. Scand. J. Clin. Lab. Invest. 1957 a, *9*, 311—312.

Reichard, H.: Ornithin-carbamyl transferasebestämming i serum. Nord. Med. 1957 b, *58*, 1016—1018.

Reichard, H.: Ornithine-carbamyl transferase in dog's serum on intravenous injection of enzyme, choledochus ligation and carbon tetrachloride poisoning. J. Lab. Clin. Med. 1959, *53*, 417—425.

Reichard, H.: Ornithine carbamyl transferase activity in human serum in diseases of the liver and the biliary system. J. Lab. Clin. Med. 1960, *57*, 78—87.

Reitman, S. & Frankel, S.: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Path. 1957, *28*, 56—63.

Rudolph, L. A., Schaeffer, J. A., Dutton, R. E. and Lyons, R. H.: Serum glutamic oxalacetic transaminase in experimental tissue injury. J. Lab. Clin. Med. 1957, *49*, 31—40.

Ticktin, H. E., Ostrow, B. H. & Evans, J. M.: Clinical applications of serum glutamic-oxalacetic transaminase. 1957, GP *XV* 81—84.

Wakim, K. G. & Fleisher, G. A.: The effect of experimental cerebral infarction on transaminase activity in serum, cerebrospinal fluid and infarcted tissue. Proc. Staff Meet. Mayo clin. 1956, *31*, 391—399.

White, A. A. & Hess, W. C.: Some alterations in serum enzymes in progressive muscular dystrophy. Proc. Soc. Exper. Biol. Med. 1957, *94*, 541—544.

Wretlind, B., Orstadius, K. & Lindberg, P.: Transaminase and transferase activities in blood plasma and in tissues of normal pigs. Zbl. Vet. Med. 1959, *6*, 963—970.

Wróblewski, F. & LaDue, J. S.: Serum glutamic oxalacetic aminopherase (transaminase) in hepatitis. J. A. M. A. 1956 a, *160*, 1130—1134.

Wróblewski, F. & LaDue, J. S.: Serum glutamic pyruvic transaminase in cardiac and hepatic disease. Proc. Soc. Exper. Biol. Med. 1956 b, *91*, 569—571.

Wróblewski, F.: The significance of alterations in serum enzymes in the differential diagnosis of jaundice. Arch. Int. Med. 1957, *100*, 635—641.

SUMMARY

The levels of glutamic-oxaloacetic transaminase (G.O.T.), glutamic-pyruvic transaminase (G.P.T.), and ornithine-carbamyl transferase (O.C.T.) were studied in different organs from healthy dogs. In the liver the activity of G.O.T., G.P.T., and O.C.T. was high. In the heart the G.O.T. level was high, the G.P.T. level lower, and the O.C.T. level very low. The skeletal muscles were found to have a very high G.O.T. level and very low levels of G.P.T. and O.C.T.

The plasma-enzyme activities were determined in healthy dogs. Mean values ($\bar{x}$), standard deviations (s. d.), and the number of dogs (n) are tabulated below.

		$\bar{x}$		s. d.	n
	G.O.T.	17.8	±	6.2	166
over 6 months	G.P.T.	20.6	±	8.4	143
under 6 months	G.P.T.	10.3	±	4.3	22
	O.C.T.	2.2	±	1.6	159

A close correlation could be demonstrated between the values for G.P.T. and O.C.T. These two enzymes have their highest activity in the liver and should therefore be useful in the diagnosis of liver damage; together with G.O.T. they should also be of value in the differential diagnosis between hepatic damage and lesions of the heart and muscles.

ZUSAMMENFASSUNG

Transaminase- und Transferaseaktivität der Plasma und verschiedener Geweben beim Hunde.

Die Enzymkoncentrationen von GOT (Glutaminsäure-Oxalessigsäure Transaminase), GPT (Glutaminsäure-Pyrotraubensäure Transaminase) und OCT (Ornithin Karbamyl Transferase) in verschiedenen Organen von normalen Hunden wurden untersucht. Die Leber enthält grosse Mengen von GOT, GPT und OCT. Das Herz enthält soeben grosse Mengen von GOT, aber weniger GPT und sehr wenig OCT. In der Skelettmuskulatur ist GOT reichlich vorhanden, der Gehalt an GPT und OCT ist dagegen sehr klein.

Die Enzymkoncentrationen in der Plasma wurden für normale Hunde bestimmt. Die Mittelwerte ($\bar{x}$), Standard-Abweichungen (s. d.) und die Anzahl der Hunden (n) sind wie folgt angegeben:

		$\bar{x}$		s. d.	n
	GOT	17.8	±	6.2	166
über 6 Monaten	GPT	20.6	±	8.4	143
unter 6 Monaten	GPT	10.3	±	4.3	22
	OCT	2.2	±	1.6	159

Eine ausgeprägte Korrelation zwischen den Werten für GPT und OCT konnte nachgewiesen werden. Da diese zwei Enzymen die grösste Aktivität in der Leber entfalten, können bei der Diagnostik der Leberbeschädigungen von Bedeutung sein und können zusammen mit GOT die Differential-Diagnose zwischen den Leberbeschädigungen einerseits und Herz- bzw. Skelettmuskelbeschädigungen anderseits, erleichtern.

SAMMANFATTNING

Transaminas- och transferasaktiviteten i blodplasma och olika vävnader hos hund.

Enzymkoncentrationerna av GOT (glutaminsyre-oxalättiksyretransaminas) GPT (glutaminsyre-pyrodruvsyretransaminas) och OCT (ornithin carbamid-transferas) har undersökts i olika organ från normala hundar. Levern innehåller stora mängder av såväl GOT, GPT som OCT. Hjärtat har höga koncentrationer av GOT, mindre av GPT och mycket lite OCT. Skelettmuskulaturen är mycket rik på GOT men mycket fattig på GPT och OCT.

Enzymkoncentrationerna i plasma har bestämts för normala hundar. Nedan anges medelvärdena ($\bar{x}$), standardavvikelser (s. d.) och antalet hundar (n)

		$\bar{x}$		s. d.	n
	GOT	17.8	±	6.2	166
över 6 mån.	GPT	20.6	±	8.4	143
under 6 mån.	GPT	10.3	±	4.3	22
	OCT	2.2	±	1.6	159

Stark korrelation kunde visas mellan värden för GPT och OCT. Dessa två enzymer har sin största aktivitet i levern och bör därför äga diagnostisk betydelse vid leverskador och tillsammans med GOT vara till hjälp vid differentialdiagnostiseringar mellan dessa och hjärt- och muskelskador.

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