

TRANSAMINASE ACTIVITY IN SERUM, URINE, AND
CEREBROSPINAL FLUID OF NORMAL AND DISEASED DOGS

by

CLAIR M. HIBBS

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D. V. M., University of Missouri, 1953

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School of Veterinary Medicine

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INTRODUCTION

Transaminases are intracellular enzymes and may be present in blood from normal individuals. Quantitative determinations of these enzymes have been used extensively as a laboratory test in human medicine to detect cellular destruction. This laboratory procedure has recently attained increased utilization in veterinary medicine with the development of kits of prepared reagents. Normal serum transaminases have been reported in the dog but very few reports have been made on the significance of these enzymes in urine and cerebrospinal fluid in this species. Most of the transaminases normals reported have been a result of one test from each of several individuals.

One purpose of this research was to establish normal oxalacetic and pyruvic transaminases in blood, urine, and cerebrospinal fluid of the dog using commercially produced reagents. No literature was found reporting the use of the transaminases in canine distemper or nephritis of the dog. Other purposes were to evaluate these enzymatic tests in dogs with natural infection of canine distemper and associated central nervous symptoms, and artificially produced nephritis.

Establishment of the normal is important in order to study the abnormal. Canine distemper and central nervous symptoms are often observed in dogs. Nephritis is also a common disease of dogs. Therefore, it is important for the veterinary clinician and the research worker to know the value of these enzymatic tests associated with these diseases.

REVIEW OF LITERATURE

Chemistry of Reaction

The transfer of an amine group from an amino acid to an α -keto acid to form a different amino acid is one of the means of synthesizing amino acids in the intermediate metabolism of animals and plants. This process is called transamination, and the enzymes that catalyze this reaction are called transaminases (Gurtler and Richter, 1959).

Braunstein and Kritzmann (1937), as reported by Green et al. (1945), discovered the process of transamination in animals and plants. These same authors (1937), as reported by Gurtler and Richter (1959), indicated that this process needed the presence of a dicarboxylic acid as a donor or acceptor of the amino group.

Schlenk and Snell (1945), as reported by Gurtler and Richter (1959), hypothesized that pyridoxal phosphate functions as a coenzyme for this reaction. O'Kane and Gunsalis (1947), as reported by Karmen et al. (1955), confirmed that pyridoxal phosphate acted as the coenzyme in the transaminase system.

Cohen and Hekhuis (1941) believed that there were three separate reactions in the transamination in tissues:

Reaction (1) Glutamic acid + oxalacetic acid \rightleftharpoons α -ketoglutaric acid + aspartic acid

Reaction (2) Glutamic acid + pyruvic acid \rightleftharpoons α -ketoglutaric acid + alanine

Reaction (3) Aspartic acid + pyruvic acid \rightleftharpoons oxalacetic acid + alanine.

They felt that analytical determination of reaction (3) would not be reliable. Most of the enzymatic determinations that have been utilized are the glutamic oxalacetic transaminase (GOT) of reaction (1) and the glutamic pyruvic transaminase (GPT) of reaction (2).

Enzyme Specificity

Cohen and Hekhuis (1941) indicated that the rate of reaction of glutamic, oxalacetic, and pyruvic acids was very slow with other amino acids. The authors believed these amino acids were not very important in intermediary metabolism. The GOT enzyme was reported to be highly specific by Green et al. (1945) and they noted that no other amino acid was found to be as active as a donor to α -ketoglutaric acid as glutamic acid. These workers found that the following chemicals could not replace aspartic acid: alanine, leucine, serine, and methionine. They also reported that glutamine, pyrrolidinecarboxylic acid could not replace glutamic acid. Mesoxalic acid could replace oxalacetic acid when both were present. Green et al. (1945) also found the glutamic alanine of pig heart to be quite stable. In the reaction between alanine and α -ketoglutaric acid, aminobutyric acid would react but at slower speed. Some chemicals that would not react were N-methylalanine, phenylalanine, valine, serine, methionine, leucine, α -aminovaleric acid, cysteic acid, and α -alanine. α -ketobutyric acid and mesoxalic acid could replace the pyruvic acid in the reaction between glutamic acid and pyruvic acid. In this reaction glutamic acid could not be replaced by cysteic acid,

glycylcysteine, glutathione, pyrrolidonecarboxylic acid, acetylglutamic acid, leucine, methionine, glutamine, tyrosine, threonine, α -aminocaproic acid, lysine, phenylalanine, cystine, valine, and hydroxyproline.

It was reported by Cammarata and Cohen (1950) that 22 amino acids in addition to alanine, aspartic acid, and glutamic acid could participate in the transaminase reaction. Each transamination appeared to be due to a different transaminase. These reactions were accelerated by pyridoxal phosphate and inhibited slightly by ammonia.

Beaton et al. (1957) concluded that liver alanine-glutamic transaminase activity was related to the direction of protein metabolism. In the study of kwashiorkor and serum transaminase, Baron (1960) found GOT increased in undernourished adults, but the GPT increase was slight. This author postulated that there were two opposing factors that could affect serum enzyme levels in protein deficiency: (1) deficient protein intake could affect the synthesis of serum enzymes since enzymes are protein, and (2) tissue wasting and cellular destruction could result in an increased level of serum enzymes. It was also reported that normal Europeans probably have higher enzyme values than normal Nigerians.

Berezowskaya and Smirnova (1956) studied rats fed 3 per cent protein diet and found a decrease in transamination of pyruvic and oxalacetic acid as compared to normal rats. This alteration could be seen after four or five days on the low protein diet. The velocity of transamination between alanine and ketoglutaric acid was reduced by 30 per cent and the velocity decreased 25 per cent

between asparagine and ketoglutaric acid as compared to normal rats.

Stability

Green et al. (1945) stated that pig heart frozen in dry ice maintained transaminase activity indefinitely. LaDue et al. (1954) found that serum stored in the refrigerator one to ten days was satisfactory for transaminase determination. Karmen et al. (1955) stored serum samples 10 minutes to 96 hours at room temperature and for one to two weeks under refrigeration of 0 to 5° C without observing any change in transaminase activity. The same workers reported that freezing or lyophilizing serum did not cause any alteration in the enzyme activity. They also reported that no enzymatic alteration occurred in serum subjected to a temperature of 56° C for 25 minutes. However, in serum heated to 100° C for 10 minutes there was a 10 per cent decrease in enzyme activity. Steinberg et al. (1956) reported little or no decrease in enzyme activity after serum had been stored in the refrigerator three weeks, and that serum stored at 4° C for four months contained 60 per cent of its original enzyme activity. Steinberg and Ostrow (1955) found that serum could be stored in the refrigerator for three weeks without a decrease in its enzyme activity. Gurtler and Richter (1959) stored serum at 0 to 5° C for two weeks without significant loss of activity. They also noted that storage of serum up to four days at room temperature did not influence enzymatic activity.

In contrast, Dubach (1957), as reported by Gurtler and Richter (1959), found a considerable decrease in activity after one-day storage of serum below room temperature. This same author reported a temperature of 18° C caused a serum glutamic oxalacetic transaminase (SGOT) decrease from 134 to 70 units after 21 hours. After incubation at 58° C, Gurtler and Richter (1959) observed a decrease from 235 units to 200 units and from 120 units to 106 units. Chinsky et al. (1957), as reported by Gurtler and Richter (1959), reported almost identical stabilities of GOT and GPT. While this storage may or may not cause a decrease in enzyme activity, it has been reported that a temperature rise from 25° C to 37° C during determination of the enzyme will double the GOT values (Bowers et al., 1958).

Comparison of Serum, Plasma, and Hemolyzed Blood GOT

Steinberg and Ostrow (1955) noted that hemolysis of human red blood cells caused an approximate eightfold increase in GOT activity. Steinberg et al. (1956) noted, while studying human transaminases, that the presence of lysed red blood cells in the serum resulted in greater GOT activity. These workers noted no significant alteration of transaminase values of plasma from heparinized or oxalated blood, and no significant changes were found in serum collected at 9:30 a.m., 11:30 a.m., and 2:30 p.m. Karmen et al. (1955) reported that human whole blood hemolysates contained about ten times the amount of GOT as normal serum. They noted no significant difference in transaminase levels of serum or plasma from oxalated, citrated, or heparinized blood. Baron

(1960) reported that hemolysis caused very little alteration in either GOT or GPT activity.

Gurtler and Richter (1959) studied the effect of hemolysis on SGOT activity in 5 horses. Hemolyzed whole blood had approximately 50 per cent more SGOT activity than its corresponding serum. These workers also noted that the GOT was slightly higher in serum than in corresponding plasma. Five corresponding sera, plasma, and hemolyzed whole blood samples from cattle were studied. The SGOT activity was slightly higher than the plasma GOT activity. Hemolyzed whole blood contained about one-half as much GOT as did the serum. Hemolyzed whole blood samples from four pigs contained about ten times greater GOT activity than the corresponding serum. The plasma of one pig contained higher GOT activity than that of the corresponding serum, but the plasma from two other pigs contained less GOT activity than the corresponding serum. Gurtler and Richter (1959) studied a single human sample and found SGOT to be 21 units, the plasma GOT 24 units, and the hemolyzed whole blood 127 units. Their work pointed out the necessity of collecting blood serum or plasma in a manner which would minimize hemolysis.

Methods of Determining Enzymatic Activity

Karmen et al. (1955) studied SGOT and serum glutamic pyruvic transaminase (SGPT) using paper chromatography. This method was sensitive, simple, and there was no loss of glutamate during the incubation period. Paper chromatography was utilized for both reactions and the results were reported in micro-moles of glutamate

produced per milliliter of serum per hour at 37° C.

Karmen (1955) developed a method for determining GOT activity of human serum by reducing the oxalacetate formed in the transaminase reaction with diphosphopyridine nucleotide (DPNH). The progress of this reaction was followed spectrophotometrically at 340 m μ using a Beckman model DU spectrophotometer. The results were reported in units per milliliter per hour, one unit being the decrease in optical density of 0.001 at room temperature. Wroblewski and LaDue (1956) used the same procedure as Karmen (1955) for determining GPT activity in human serum.

Tonhazy et al. (1950) developed a colorimetric method of determining glutamic aspartic transaminase in tissues.

Cabaud et al. (1956) measured GOT activity in serum of patients by converting the oxalacetate to pyruvate using aniline citrate. The pyruvate reacted with 2,4-dinitrophenyl hydrazine and a strong alkali to form a hydrazone, the quantity of which was determined colorimetrically at 490 m μ with the blank set at 100 per cent transmittance. One unit equalled the formation of 1 gamma of pyruvate under conditions of the test. The same workers checked 35 sera and found a 16 per cent error. Wroblewski and Cabaud (1957) described a similar procedure for determining SGPT.

Steinberg et al. (1956) described a method for assaying SGOT which was similar to that of Karmen's (1955). The change in optical density was measured at 340 m μ at 25° C. One unit equalled a decrease in optical density of 0.001 per centimeter of light path per minute of incubation at 25° C.

Reitman and Frankel (1957) described a colorimetric method for determining SGOT and SGPT which was a simplification of the method of Cabaud et al. (1956). The incubation period for SGOT was one hour at 40° C and for SGPT 30 minutes at 40° C. The optical density was measured at 505 m μ using water as the reference standard. Any wavelength between 490 and 530 m μ could be used as long as it was kept constant. The readings were based upon the difference in absorption of a-ketoglutarate, oxalacetate, pyruvate, and 2,4-dinitrophenylhydrazine at 505 m μ . The ratio of pyruvate to a-ketoglutarate was related to transaminase units. One unit was similar to one Karmen unit.

Bowers et al. (1958) described what was called a simple and accurate method of determining SGOT and SGPT activity. The general method of Karmen (1955) was used except the tests were incubated at 37° C and read at 355 m μ . These authors did not explain what constituted a unit.

King (1958), as reported by Daly and Jordan (1959), used the technique of Reitman and Frankel (1957) in which one unit equaled the activity in 100 ml of serum which would convert one micro-mole of amino acid to a keto acid in one hour at 37° C.

Serum Transaminase Levels in Normal Animals

A compilation of SGOT and SGPT values in normal animals is shown in Table 1. In the dog, Lemley-Stone et al. (1955) reported a normal mean SGOT level of 39 units with standard deviation of 3.5 units per milliliter. Nydick et al. (1955) found the normal SGOT range to be 8 to 40 units per ml. Wakim et al. (1956) found

Table 1. Normal transaminases reported in animals.

Author	Method	Animal	No. :	Units SGOT		No. :	Units SGPT	
				: Animals	: Serum or plasma		: Animals	: Method
Lemley-Stone et al. (1955)	Karmen (1955)	dogs	19	39±3.5				
Wakim et al. (1956)	Karmen (1955)	dogs	33	1.05±0.17 uM/mL/hr				
Seigle and Bing (1956)	Karmen (1955)	dogs		15-39				
Ray (1958)	Reitman-Frankel (1957)	dogs	19	mean 18.60 12-48		19	Reitman-Frankel (1957)	mean 22.5 10-62
Cornelius et al. (1959)	Reitman-Frankel (1957)	dogs	25	22.7±5.4			"	21.8±6.2
"	"	horses	36	165±33.8		11	"	11±3.8
"	"	cows	21	43.8±5.7		16	"	19.7±12.6
"	"	calves	8	23.6±3.7		8	"	7.8±3.2
"	"	pigs	10	31.1±14.1		10	"	27.3±7.8
Gurtler and Richter (1959)	Cabaud (1956)	horses	63	mean 260 range 163-367		23	Wroblewski and Cabaud	mean 9 range 0-20

Table 1 (concl.).

Author	Method	Animal	No. : : Animals	Serum or plasma	SGOT	No. : : Animals	Serum or plasma	SGPT	Method	Serum or plasma
Gurtler and Richter (1959)	Cabaud (1956)	cows	66	mean 90 range 56-165	mean 90 range 56-165	22	Wroblewski mean 30 and Cabaud range 9-64			
"	"	pigs	25	mean 51 range 10-98	mean 51 range 10-98	21	" mean 47 range 17-72			
Blinco and Dye (1958)	Cabaud (1956)	lambs	20	mean 128 range 97-191	mean 128 range 97-191					
"	"	lambs	21	mean 56 range 22-160	mean 56 range 22-160					
Kuttler and Marble (1958)	Cabaud (1956)	calves	45	56±17	56±17					
"	"	calves ¹	21	112±64	112±64					
"	"	lambs	21	56±31	56±31					
"	"	lambs ²	4	208±192	208±192					

¹ Normal calves from WMD infected herd

² Normal lambs from WMD infected flock

a SGOT range of 0.70 to 1.34 $\mu\text{M}/\text{ml}/\text{hr}$. Cornelius et al. (1959) reported the normal mean of canine SGOT to be 22.7 units and a standard deviation of 5.4 units. The same author also found the mean SGPT to be 21.8 units and a standard deviation of 6.2 units.

Blinco and Dye (1958), working with lambs, reported a normal SGOT range in one group of lambs of 97 to 191 units and in another group found a normal SGOT range of 22 to 160 units. The normal SGOT range in lambs of 19 to 99 units was reported by the same authors. Kuttler and Marble (1958) also reported the normal SGOT of calves and lambs. They found the mean SGOT levels of calves from a so-called "disease free herd" to be 56 units with a standard deviation of 17 units. However, the mean SGOT level of normal appearing calves from a known white muscle disease (WMD) affected herd was 112 units with a standard deviation of 64 units. The normal SGOT in a "disease free flock" of lambs was found to be 56 units with a standard deviation of 31 units. Normal appearing lambs from a known WMD affected flock had a mean SGOT of 208 units with a standard deviation of 192 units.

Compagnucci (1959) found the normal SGOT range in the bovine to be 48 to 83 units and the normal SGPT range was 9.2 to 20.4 units. The normal SGOT in 34 cattle studied by Gould and Grimes (1959) was 38 to 102 Reitman-Frankel units with a mean of 64.9 units. Gurtler and Richter (1959) reported the normal range SGOT in the ox to be 56 to 165 units while the SGPT was 9 to 64 units. Cornelius et al. (1959) gave the normal mean SGOT of Holstein cows as 55.8 μg pyruvate/ml and 43.8 Sigma-Frankel units. In calves, the same workers found a normal mean SGOT of 25 μg

pyruvate/ml and 23.6 Sigma-Frankel units. The normal mean SGPT in cows was 16.2 ug pyruvate/ml and 19.7 Sigma-Frankel units. The calves' normal mean SGPT was recorded as being 2 ug pyruvate/ml and 7.8 Sigma-Frankel units.

Cornelius et al. (1959) reported the normal mean SGOT of pigs to be 31.1 Sigma-Frankel units while the SGPT was 27.3 Sigma-Frankel units. The standard deviations were 14.1 units and 7.8 units, respectively. Gurtler and Richter (1959) found the normal SGOT range of pigs to be 10 to 98 units with a mean of 51 units and a standard deviation of 24.9 units. They also reported the normal SGPT range of pigs to be 17 to 72 units and a mean of 47 with a standard deviation of 12 units.

Gurtler and Richter (1959) reported the normal SGOT range in horses to be 163 to 367 units with a mean of 260 units and a standard deviation of 42.8 units. The normal range of SGPT was 0 to 20 units with a mean of 9 units and a standard deviation of 5.9 units. Cornelius et al. (1959) found the mean SGOT of the equine to be 158 ug pyruvate/ml with a standard deviation of 37.4 ug pyruvate/ml and 165 Sigma-Frankel units with a standard deviation of 33.8 Sigma-Frankel units. The same workers reported the normal mean SGPT of the equine of 8.0 ug pyruvate/ml with a standard deviation of 5.9 ug pyruvate/ml and 11.0 Sigma-Frankel units with a standard deviation of 3.8 Sigma-Frankel units.

Urine

Steinberg and Ostrow (1955) reported that normal human urine contained less than 1 unit/ml of GOT. Dunn et al. (1958) found

no transaminase activity in the urine of dogs and postulated that the enzyme molecule was too large to pass through the glomerulus of the kidney or that the tubules were able to completely absorb the enzyme. Chinskey et al. (1956) reported insignificant amounts of transaminase activity in urine of normal humans. The same workers found that patients with high SGOT had significant levels of urine glutamic oxalacetic transaminase (UGOT). The UGOT was not nearly as high as the SGOT.

Cerebrospinal Fluid

Wakim and Fleisher (1956) found normal cerebrospinal fluid glutamic oxalacetic transaminase (CFGOT) in dogs to be approximately one-half that of their normal SGOT. Lending et al. (1959) reported that normal human CFGOT to be from 1 to 10 units/ml/min. and that enzymes were higher during the first 24 hours of life. Green et al. (1957), as reported by Arst (1959), concluded that human cerebrospinal fluid had about one-half as much GOT activity as the corresponding serum. These authors reported the normal human CFGOT was from 2 to 7 units. Using the Reitman-Frankel method, Arst (1959) reported the normal human CFGOT to be between 0 and 39 units. This range was derived from 133 normal individuals. Wakim and Fleisher (1956) found a mean level of GOT in cerebrospinal fluid of 0.66 $\mu\text{M}/\text{ml}/\text{hr}$. in the dog.

Tissue Transaminases

The transaminases in tissues of white rats have been calibrated by Cohen and Hekhuis (1941). These workers found that

heart muscle had the largest amount of GOT, and skeletal muscle the second largest. These were followed in order by brain, liver, kidney, testis, lung, and spleen. These same workers reported that what is now called GPT activity was much more rapid in the liver than in other tissues examined. The work of Cornelius et al. (1959) also indicated that GOT was greatest in heart muscle, closely followed by skeletal muscle in most animals studied. Their work showed a comparatively large amount of GPT activity in the liver of the dog. The equine had a very high GOT in the pancreas. The same tissues showed a great deal of variation between individuals. Wroblewski et al. (1956), as reported by Gurtler and Richert (1959), found that in man the GOT activity was greatest in heart muscle followed by liver and then skeletal muscle. The GOT level in the kidney tissue closely approximated that of skeletal muscle. The largest amount of GOT activity in the tissues of rats studied by Awapara and Seal (1952) (Gurtler and Richter, 1959) was in heart muscle followed in order of importance by skeletal muscle, kidney, brain, liver, and testicle. From the report of Cammarata and Cohen (1950), LaDue et al. (1954) calculated that if 1 gram of dried pig heart was diluted to 6 liters, there would be 400 GOT units/ml. Dunn et al. (1958) estimated the extracellular fluid pool of a dog to contain about 30,000 units. A 2-kilogram pig heart was estimated by Green et al. (1958) to contain approximately 750,000 GOT units. Fleisher and Wakim (1956) reported GPT practically absent in the brain of the normal dog.

The literature cited indicated that the largest amount of GOT activity was found in the heart muscle, skeletal muscle, and liver. Other tissues showed a much smaller activity.

Enzymatic Alteration Due to Surgery

No elevation of SGOT was found in 50 per cent of post operative patients by Watanobe et al. (1957). Some of the post operative patients had a slight SGOT elevation while one had a marked increase in enzyme activity. Sickert et al. (1956) reported slight elevation of SGOT as a result of surgery. Rudolph et al. (1957) proposed that necrosis of any type of tissue would elevate the SGOT, the degree being directly proportional to the amount of tissue damaged.

Enzymatic Alteration Due to Heart Disease

Rudolph et al. (1957) produced experimental infarction of the heart of dogs with resulting SGOT findings of 45 to 608 units.

LaDue et al. (1954) studied 30 patients with transmural myocardial infarction. They found SGOT to be normal 3 hours after the onset of pain. Twelve hours after onset of pain, the SGOT level had risen to as high as 500 units. The peak levels of SGOT were from 100 to 6000 units. In this group of patients, SGOT returned to normal by the sixth day. Increase in transaminase activity was reported in clinical cases of human arteriosclerotic heart disease and recent myocardial infarction. Chinsky et al. (1956) reported myocardial infarction caused an elevated SGOT about 6 hours after onset of chest pain. The peak occurred 24

hours after the first chest pain and returned to normal level on the third to the sixth day. Wroblewski and LaDue (1956) failed to find much increase in SGPT as a result of myocardial infarction. Agress et al. (1955) introduced plastic spheres into coronary ostia of experimental dogs to artificially produce myocardial infarction. Electrocardiographic S-T depression was considered to be the time of injury by the spheres. The peak rise in SGOT was found to be 9 to 23 hours after time of injury. SGOT values 20 to 30 times normal were obtained by the procedure. Members of the research group, separate from the ones determining the transaminases, estimated the amount of infarction at autopsy in terms of per cent of gross total heart tissue. They concluded that the SGOT levels were directly proportional to the amount of gross damage due to infarction. The authors thought the estimation of amount of infarction was accurate to within 5 to 10 per cent.

Karmen et al. (1955) reported transaminase increase due to arteriosclerotic heart disease and recent myocardial infarction. Kattus et al. (1956) studied 5 convalescent patients with myocardial infarctions and found the transaminases to be essentially normal. The same authors reported 4 patients with congestive heart failure and 4 patients with acute benign pericarditis to be within the normal range. Acute myocardial infarction was found to give peak transaminase levels at 24 to 36 hours with a return to normal by the fifth day after clinical onset. There was an unexplained delay in the rise of SGOT from 6 to 12 hours after onset.

Steinberg and Ostrow (1955) studied 24 cases of myocardial necrosis and found that in all but two cases the SGOT was elevated above 40 units within 48 hours after onset of pain. Peak readings of 54 to 308 units were recorded. These occurred 36 hours after the first pain. They reported no significant SGOT elevations in cases of myocardial ischemia, angina pectoris, uncomplicated cardiac failure, and pericarditis. The increase in SGOT due to myocardial necrosis was presumed to be a result of the release of the enzyme from damaged heart muscle cells. Due to the rapid rate of disappearance of the enzyme, despite its stability, the authors postulated that there was some mechanism which removed it from the blood. No further explanation was given.

Watanobe et al. (1957) found no SGOT elevations in cases of mild benign idiopathic pericarditis, congestive heart failure, aortic stenosis, healed myocarditis, mitral stenosis with atrial flutter, subacute bacterial endocarditis, and cardiac neurosis. Slight elevations were reported in cases of rheumatic pericarditis and pulmonary infarction associated with congestive heart failure. Marked elevations were found in cases of severe idiopathic pericarditis and terminal cyanosis and collapse due to valvular heart disease.

Enzymatic Alterations Due to Skeletal Muscle Pathology

Skeletal muscle was found to be second to cardiac muscle in GOT activity (Cohen and Hekhuis, 1941; Wroblewski et al., 1956; Cornelius et al., 1959; Awapara and Seale, 1952, as reported by Gurtler and Richter, 1959). Sickert et al. (1956) noticed

elevated SGOT levels in cases of dermatomyositis, muscular dystrophy, and gangrene of the toes of human patients. The SGOT was not elevated in progressive muscular dystrophy. In contrast, Watanobe et al. (1957) did not find SGOT elevated in dermatomyositis. Marked elevations were reported in cases of muscular dystrophy and paroxysmal myoglobinuria. White and Hess (1957) studied cases of muscular dystrophy in the human and found that SGOT levels increased. Eight children with muscular dystrophy were found to have a SGOT mean of 2.59 $\mu\text{M}/\text{ml}/\text{min}$. as compared to normal children with a SGOT mean of 0.85 $\mu\text{M}/\text{ml}/\text{min}$. Correspondingly, 9 cases of muscular dystrophy in adults had mean SGOT values of 0.86 $\mu\text{M}/\text{ml}/\text{hr}$. with the normal adult mean SGOT being 0.65 $\mu\text{M}/\text{ml}/\text{hr}$.

White muscle disease in calves and sheep corresponds somewhat to muscular dystrophy in humans. Kuttler and Marble (1958) utilized this similarity and studied the SGOT levels of WMD in calves and lambs. This study utilized the naturally occurring disease as well as cases that were artificially produced by feeding a diet of cod liver oil and milk. In artificial cases of WMD, the first SGOT increase was noted on the eleventh day. Clinical signs were evident on day 23. Another case had initial elevation of SGOT on day 21, but clinical symptoms did not appear until day 27. Two affected lambs were treated with 600 gm. d-alpha tocopherol acetate followed by 450 mg. the next 2 days. The animals returned to normal clinically and the SGOT dropped in those animals that were checked. The mean SGOT value of the 14 lambs with artificially induced WMD was 4,498 units \pm 2,213 units. Seventeen

field cases of WMD had a mean SGOT level of 2,574 units \pm 2,692 units. These same researchers found that five field cases of WMD in calves had a mean SGOT of 524 \pm 236 units. No significant difference was found in SGOT of the cardiac and skeletal type of the disease. Signs of illness usually appeared in lambs when the SGOT level reached 2,000 units, and in calves when the SGOT level reached 3,000 units. These authors noted that some apparently normal animals in infected flocks of sheep had high GOT levels.

Seventeen lambs with WMD confirmed at autopsy were studied by Blinco and Dye (1958). Lambs with WMD had SGOT range of 687 to 3,460 units. The 4 calves checked had a SGOT range of 295 to 2,360 units. These authors reported a more pronounced increase in SGOT in lambs than in calves. It was also noted that the SGOT level increased with the severity of the disease.

Kuttler and Marble (1959) used the same method of feeding cod liver oil and milk to lambs to produce WMD. They recorded a peak of 8,116 units in one lamb. Peaking of the SGOT level occurred between 18 and 25 days after starting the animals on this diet. Samples of blood were taken at 7 and 14 days after feeding the same disease-producing diet to 418 lambs (Swingle et al., 1959). It was reported that of 77 lambs with WMD, SGOT units of over 400 units occurred in 74 lambs. They indicated that SGOT predicted accurately the onset of WMD before clinical signs were manifested.

Transaminase Activity Associated with Liver Disease

As reported earlier in the review, Cohen and Hekhuis (1941) indicated the importance of SGPT in the liver of the rat. They felt that there was more than one transaminating enzyme in the liver. LaDue et al. (1954) noted high SGOT values in jaundiced human patients with acute liver disease. Later, Karmen et al. (1955) reported that transaminase activity was higher than normal in acute hepatitis. Kattus et al. (1956) found that two cases of viral hepatitis reached serum aminopherase peaks at 13 and 18 days after onset and returned to normal on the 32nd and 33rd days. The SGPT was not appreciably elevated in diseases that did not involve the liver (Wroblewski and LaDue, 1956). These authors surveyed 12 human patients with viral infectious hepatitis and found an elevated SGPT. Fifteen human patients with metastatic cancer of the liver were studied. Five had elevated SGPT and 12 had elevated SGOT levels.

Molander et al. (1957) studied the following human diseases and corresponding SGOT levels: 81 patients with Laennec's cirrhosis, 13 to 286 units; 7 biliary cirrhosis, 57 to 330 units; one obstructive jaundice, 320 units; 20 viral hepatitis, 540 to 1890 units; and also reported that 2 hepatitis patients of Molander et al. (1955) had 27,840 and 12,300 units. The patients with obstructive jaundice had a drop in SGOT following drainage of the bile duct. Patients with viral hepatitis of 3 to 4 weeks duration had little SGOT elevations. It was their belief that the severest liver conditions had the highest SGOT levels.

Watanobe et al. (1957) reported SGOT levels of 100 to 200 units in cases of biliary obstructive jaundice, but with cellular destruction the SGOT were found in non-icteric cirrhosis of the liver or congenital hemolytic jaundice. Slight SGOT elevation was recorded in quiescent non-icteric cirrhosis of the liver. Marked increase in SGOT was found in cases of viral hepatitis, obstructive jaundice, liver cirrhosis with jaundice, and metastatic cancer of the liver. Chinskey et al. (1956) reported that in human jaundice, the serum bilirubin tended to follow serum transaminase levels.

Liver damage caused by administering carbon tetrachloride to animals (Cornelius et al., 1959) caused an elevation in SGOT of pigs, horses, dogs, and calves. Peaks of 2800 units of SGOT and over were recorded on day 1 in the 2 pigs studied. The dog died but had a SGOT of 2400 units at that time. Other animals examined did not have SGOT levels over 850 units. There was not much change in SGPT levels in this same group of animals with the exception of the dog whose SGPT reached a peak of 2760 units on the second day after administration of carbon tetrachloride. The highest SGPT in other animals studied was near 100 units in the calf at death. The other calf, one pig, and the horse continued to have normal SGOT levels.

Compagnucci (1959) found the SGOT in ten bovine cases of diffuse hydatidosis to be 56 to 150 units. The SGPT was reported as being 20 to 62 units. The cases of slight hydatidosis and tuberculosis were reported to have normal SGOT and SGPT levels.

Transaminase Activity Associated with Diseases of the Central Nervous System

Cases of neuritis, and patients with nerve section were reported by Sickert et al. (1956) to have normal SGOT. Dogs with carbon tetrachloride poisoning had increased CFGOT levels (Fleisher and Wakim, 1956). They reasoned that the CFGOT increase was due to cerebral damage as a result of the carbon tetrachloride followed by transfer of the enzymes from the blood to the cerebrospinal fluid and/or hemorrhage into the spinal canal during puncture.

Wakim and Fleisher (1956) injected vinylacetate into the internal carotid artery and produced cerebral infarction. The resulting mean CFGOT in the 7 dogs was 5.50 $\mu\text{M}/\text{ml}/\text{hr.}$ which represented an increase of 733 per cent above normal. The SGOT was 110 per cent above normal. Brain tissue examined after infarction had a GOT value 71 per cent below normal. The authors concluded there was no correlation between SGOT and severity of infarction while there was a direct correlation between CFGOT and damage to the brain.

Watanobe et al. (1957) found marked SGOT elevation associated with infarction of the brain but none to a slight elevation in cases of cerebral accidents. Kuttler and Marble (1958) reported no elevation in SGOT in one lamb with staphylococcal meningitis.

The extensive study of Lending et al. (1959) of GOT in cerebrospinal fluid and serum of normal and abnormal infants pointed out the importance of CFGOT determination in case of brain disease. The CFGOT range in abnormal infants was 31.6 to 855

units. This was an increase of 82 per cent. The SGOT was elevated only 18 per cent. The authors used 1 ml of cerebrospinal fluid and followed the procedure of Karman et al. (1955). The authors postulated that the increase in enzyme activity in the cerebrospinal fluid was due either to increased permeability of the blood-cerebrospinal fluid barrier or to lysis of cerebral cells and changes in permeability of cerebral cell membranes. Allgen et al. (1958) followed 400 delirium tremens and allied cases. SGOT in delirium tremens was markedly elevated. This increase was simultaneous with mental and somatic manifestations. The SGOT peak was found at the height of symptoms. SGOT readings in severe cases were 200 to 1,000 units while moderate cases had less than 300 units. As somatic and mental symptoms stopped, the SGOT returned to normal. CFGOT was checked in a few cases and found to be within the normal range. These authors also noted that patients with long periods of abstinence would develop elevated SGOT after one day of drinking. The SGPT was also increased, but not as much as was SGOT. Severe cases of delirium tremens had SGPT levels up to 200 units.

Arst (1959) concluded after studying 66 patients with central nervous system disorders that CFGOT determinations had little clinical significance. Two cases of cryptococcosis and one case of toxoplasmosis did result in CFGOT values which were above normal.

Transaminase Activity Associated with Diseases of the Kidney

Steinberg and Ostrow (1955) found no SGOT elevation in nephrosis with marked edema. Later, Watanobe et al. (1957) found no significant elevation in chronic nephritis. Slight SGOT elevations in probable renal infarction and marked elevation in infarcts of the kidney were recorded by the same workers.

Eleven cases of children with a nephrotic syndrome were studied by Stanton and Howard (1959) and found to have normal SGOT. These workers also reported normal SGOT in acute glomerulonephritis while 15 children with renal disease were found to have below normal SGOT. Kemp (1960) studied the enzyme levels of the arterial and venous blood of the kidney. He reported arteriovenous differences in GOT in the kidney of normal rabbits to be positive or zero. It was postulated this difference was due to destruction of enzymes by the normal kidney.

Rudolph et al. (1957) found that dogs with experimental multiple infarcts of the kidney had SGOT levels of 114 to 149 units. Nephrectomy elicited only slight elevation of the SGOT to 58 units.

Fate of the Transaminases

Reports have substantiated the fact that there are elevations of SGOT and SGPT in certain diseases, but very few workers have indicated what happens to the enzymes as the level returns to normal. Fleisher and Wakim (1956) indicated that GOT and GPT were eliminated from tissues at the same rate. Dunn et al. (1958)

injected autogenous muscle extract, homologous liver, and homologous heart intravenously into dogs. The serum GOT of the dog was determined and compared with the amount in the material injected. The SGOT dropped rapidly during the first 6 hours after injection of material. SGOT levels returned to normal 18 to 60 hours subsequent to injection. Disappearance of GOT from autogenous and homologous GOT extracts was similar. Strangely, the disappearance curve of bilaterally nephrectomized dogs was similar to the same dogs after injection of GOT containing material indicating little or no loss of the enzyme through the urine.

Dunn et al. (1958) found no correlation between SGOT and the concentration and excretion of GOT in the bile, although these workers did find that bile GOT was increased after injection of GOT material.

Other Diseases Studied for Alterations of Enzymatic Activity

The transaminases have been determined in various other disease conditions. Normal SGOT levels were found in cases of pulmonary infarction and acute pneumothorax by Steinberg and Ostrow (1955). Wroblewski and LaDue (1956) reported that leukemia or lymphomatous disease might alter the SGOT but not the SGPT. In a review, Mason and Wroblewski (1957) cited literature which stated that SGOT levels were not related to any of the following: shock, blood pressure, anticoagulants, digitalis, quinidine, age, sex, color, temperature, weight, sedimentation rate, white blood cell count, or oliguria.

In addition to the SGOT values for human diseases listed previously by Watanobe et al. (1957), these authors found no elevation of SGOT in cases of pulmonary infarction, various forms of cancer, pneumonia, subphrenic abscess, aneurysm, and diabetes. Karmen et al. (1955) noted increased SGOT in human lymphomatous disease, extensive rhabdomyosarcoma, and acute leukemia.

While Kuttler and Marble (1958) were studying WMD in calves and lambs, they also noted that 6 castrated lambs had no significant alterations in SGOT. A lamb with photosensitization had a SGOT of 850 units and one lamb with myiasis had a SGOT of 441 units. Blinco and Dye (1958) recorded no SGOT increase in scours and only a slight increase in staphylococcal meningitis in lambs. A case with severe internal injuries had a SGOT of 82 units, while those with toxemia had SGOT levels from 42 to 188 units. Swingle et al. (1959) recorded a high SGOT in a lamb with suppurative arthritis but found lambs with bronchopneumonia had normal SGOT.

One hundred thirteen pregnant women were studied by Stone et al. (1960) during all trimesters of pregnancy, the intrapartum period, and post partum. No elevation in SGOT was reported in any of the patients and in many instances the SGOT values were lower than those of non-pregnant women.

Tonhazy et al. (1950) recorded normal SGOT in mice ill with radiation sickness. Molander et al. (1957) reported that SGOT of one patient with obstructive jaundice associated with Hodgkin's disease fell from 320 to 57 units after a T tube was placed in the common bile duct, and later dropped to 27 units following radiation therapy.

Steinberg and Ostrow (1955) reported that Dr. Andrew G. Morrow had done preliminary studies indicating that experimental intestinal infarction in dogs could cause significant SGOT elevation.

MATERIALS AND METHODS

Experimental Animals

Four groups of dogs of mixed breeding and of both sexes were used. The animals were from six weeks to two years of age. The dogs were maintained in isolation and fed a commercial dry dog food during the experiment.

The dogs in Group I were purchased in Kansas and were delivered on April 23, 1961. These dogs were divided into pens of four and vaccinated with a tissue culture canine distemper vaccine.¹ Upon challenge with intracerebral inoculation with canine distemper street virus, these dogs were found to be immune. Urine and blood were collected at varying intervals prior to intracerebral inoculation. Cerebrospinal fluid was collected just before intracerebral challenge.

This group was later inoculated with 1.0 ml of a 1:20 suspension of canine hepatitis virus. The deaths occurred during the night precluding the collection of samples.

Group II consisted of dogs raised at Kansas State University or purchased at various times from local farmers.

¹ Jen-Sal Laboratories, Kansas City, Missouri.

These dogs were unilaterally nephrectomized. A period of at least four weeks later samples were collected. Later, all dogs except control dogs were inoculated with a strain of Proteus sp. which had been isolated from a clinical case of canine nephritis. These dogs were then called Group V. The methods of inoculation were as follows: (1) one-fourth ml of a 24-hour culture in trypticase soy broth² with 0.1 per cent agar added was inoculated into the renal artery of the remaining kidney; (2) twenty ml of a saline washed culture was inoculated into the renal artery of the remaining kidney; (3) ten ml of a saline washed culture was injected into the peripheral circulation every day for seven days. All cultures used for inoculation were checked for viability of the bacteria and found to be living.

Group III consisted of dogs purchased in Nebraska. The arrival date was January 23, 1961. These dogs were vaccinated against distemper. Shortly after arrival, these dogs developed a diarrhea, nasal discharge, eye discharge, and some became comatose followed by death. Chorea-like symptoms were observed in certain dogs. When possible, samples of urine, blood, and cerebrospinal fluid were collected prior to death.

A tentative diagnosis of canine distemper was given at autopsy. This diagnosis was later confirmed by histopathology.

The dogs in Group IV were purchased in Arkansas and were delivered May 23, 1961. The dogs arrived in Manhattan apparently healthy. Since these dogs were being utilized in other research,

² Baltimore Biologic Laboratories, Baltimore, Maryland.

controls could not be collected. These dogs were divided into pens and handled in a manner similar to Groups I and III.

All dogs exhibiting central nervous disturbance were utilized. Urine, plasma, and cerebrospinal fluid were collected from each dog prior to euthanasia.

Collection of Plasma, Serum, Urine, and Cerebrospinal Fluid

All blood samples were collected from the radial, saphenous, or jugular vein using ethylenediaminetetraacetate (EDTA) as the anticoagulant. The blood for serum samples was collected in the same manner except no anticoagulant was used. All samples for analysis were either serum or plasma. Only unhemolyzed samples were utilized.

The urine was collected by catheterization or by urinary bladder tap, using an 18 gauge 1.5 inch needle. Prior to bladder tap, the skin was scrubbed with alcoholic Roccal.³

Cerebrospinal fluid was collected by flexing the head of the anesthetized dog ventrally, and locating the area just dorsal to the foramen magnum. The surface was scrubbed with alcoholic Roccal. A sterile 20 gauge one inch needle with a sterile 5 c.c. glass syringe attached was inserted into the foramen magnum. Two ml of cerebrospinal fluid were aspirated. Only clear samples were saved for testing.

The plasma, serum, urine, and cerebrospinal fluid samples were stored at -20° C. or used the day collected. The stored

³ Winthrop Laboratories, New York 18, New York.

samples were allowed to warm to room temperature before being used for transaminase determination. No samples were stored more than two weeks.

Transaminase Procedures

GOT Procedure. One ml of a standard solution of aspartic and α -ketoglutaric acids⁴ buffered to a pH of 7.5 was pipetted into a 15 ml test tube and placed in a water bath at 37° C. for at least five minutes. After the initial incubation, 0.2 ml of the unknown (plasma, serum, urine, or cerebrospinal fluid) was added to the substrate. This was mixed and placed in a 37° C. water bath for exactly one hour.

At the end of one hour incubation, 1 ml of a standard solution of 2,4-dinitrophenylhydrazine (DNPH) was added. This solution was mixed and allowed to stand at room temperature for 20 minutes. Ten ml of 0.4 N sodium hydroxide was added. The tube was inverted and allowed to stand at room temperature for at least five minutes.

This material was poured into a standard cuvette. All readings for each day were made using the same cuvette. The per cent transmittance was determined in either a Bausch and Lomb Spectronic 20 colorimeter⁵ or a Coleman Jr. spectrophotometer.⁶ The instrument was adjusted to 100 per cent transmittance at a

⁴ All reagents for GOT and GPT were furnished by Dade Reagents, Inc., Miami, Florida.

⁵ Bausch & Lomb Optical Company, Rochester, New York.

⁶ Coleman Instruments, Incorporated, Maywood, Illinois.

wavelength of 505 m μ using distilled water as the reference standard.

The number of units were read directly from a standard curve. Under the conditions of the test, one unit was equal to the formation of pyruvate-dinitrophenylhydrazone equivalent to one gamma of pyruvate.

The GPT procedure was conducted in the same manner as the GOT except the substrate used was a standard solution of α -glutaric acid and alanine buffered to a pH of 7.5. The incubation time of the unknown with the substrate was 30 minutes.

Standard Control

The calibration tubes were set up as indicated in Table 2.

Table 2. Procedure for establishing calibration curve.

Cuvette No.	: : Water	:Standard :substrate	:Calibration: : standard	SGOT : Units/ml	SGPT : Units/ml
1	0.2 ml	1.0 ml	0.0 ml	0	0
2	0.2 ml	0.9 ml	0.1 ml	22	25
3	0.2 ml	0.8 ml	0.2 ml	55	50
4	0.2 ml	0.7 ml	0.3 ml	95	85
5	0.2 ml	0.6 ml	0.4 ml	150	126
6	0.2 ml	0.5 ml	0.5 ml	215	--

One ml of DNPH was added to each of the above tubes and allowed to stand at room temperature for twenty minutes. Ten ml of 0.4N sodium hydroxide was added to each tube. The tubes were

inverted and allowed to stand at room temperature for at least five minutes. The readings were made as described in the GOT and GPT procedure. Calibration readings were plotted on single cycle semi-logarithmic paper with 84 divisors.

All reagents were of the same lot number. As each group of unknowns was tested, two control tubes were prepared. These controls were at levels representing 22 units of GOT and 25 units of GPT in one tube and 95 units of GOT and 83 units of GPT in the other tube. All points of the calibration curve were checked at periodic intervals. Standard curves were prepared for both the Bausch and Lomb Spectronic 20 and the Coleman Jr. spectrophotometers.

During the early part of this study 0.2 ml of urine was added to the reference blank and used in the procedure. This was done to check the effect that the urine pigments had on the percent transmittance at this wavelength. This did not prove to be a significant factor, so the procedure was discontinued.

RESULTS AND DISCUSSION

Serum Glutamic Oxalacetic Transaminase

The SGOT findings are summarized in Table 3.

The normal range was considered to be the mean plus or minus the standard deviation. Therefore, the normal SGOT range for the dogs studied was found to be 17 to 36.2 units in group I and 18.7 to 41.7 units in group II. Any dogs having a SGOT between 17 and 41.7 units were considered to be within the normal range.

Table 3. Summary of serum glutamic oxalacetic transaminase (SGOT).

Group No.	No. dogs	No. determinations	\bar{x}	$s_{\bar{x}}^2$	s	Range in units
I Normal	15	38	26.6	1.555	9.58	12-55
II Normal	28	103	30.18	1.129	11.50	4-66
III Distemper	7	7	38.9	8.610	22.74	12-62
IV CNS ⁴	11	11	78.0	23.10	61.00	22-162
V Nephritis	15	96	29.0	5.477	13.62	8-160

- 1 Mean SGOT in units
- 2 Standard error in units
- 3 Standard deviation in units
- 4 Central nervous symptoms

Using the confidence interval, there was a statistically significant difference between these two groups. However, there was also a difference between the results of the studies of Ray (1958) and Cornelius et al. (1959) using this same method of analysis (Table 1). Both of these workers used the same method of enzymatic determination. The normal mean SGOT found in this research was higher than the above authors, but lower than that of Lemley-Stone (1958) (Table 1).

The SGOT dog-to-dog variation in groups I and II was 9.58 units and 11.5 units, respectively. Therefore, the mean difference between the two groups was considered to be relatively normal for this method of determination.

Mason and Wroblewski (1957) reported a transient SGOT elevation one to four days following surgery. Collection of samples from all dogs in group II was not instituted for at least one month following nephrectomy. Therefore, this probably was not a

factor in the group II dogs.

Standard errors of these two groups of normal dogs were found to be small. This indicated the accuracy of this method of test.

In group III, all of the data indicated that the SGOT determination was not a reliable diagnostic aid in the detection of canine distemper. The mean SGOT was 38.9 units with a range of 16.2 to 61.6 units. This range was considered to be too near the normal to be of diagnostic value. Three of the seven dogs in this group had SGOT readings of 62 units. The other four dogs had 30 SGOT units or less.

There was a significant mean SGOT increase in the dogs with central nervous system involvement. However, the SGOT level of six of these dogs was within the normal range. The SGOT elevation of the other five dogs may have been due to one or more of the following: (1) cellular death during the course of the disease, (2) cellular destruction associated with impending death regardless of the cause, (3) accumulation of the enzymes due to reduced metabolic activity just prior to death. The standard error and standard deviation were extremely large in this group.

Although the SGOT range in group IV was wide, the mean SGOT was well within the normal. There did not seem to be a specific pattern in the SGOT of these dogs. Although there was generally a transient rise in SGOT for from one to three days in most of the dogs following the surgical procedure of inoculation, only two dogs of this group had SGOT above 66 units during this experiment (Table 4). One of these had an SGOT of 86 units the day following inoculation into the renal artery. This may have been due to the

Table 4. Transaminases of two nephritic dogs.

Date	:	SGOT	:	SGPT	:	UGOT	:	UGPT
<u>Dog #18H</u>								
12-31-60				unilateral nephrectomy				
2- 8-61		39		42		31		24
4-13-61		33		42		62		46
4-22-61		24		27		25		26
4-22-61		inoculated with 20 ml <u>Proteus</u> sp. via renal artery						
4-23-61		54		44		14		16
4-24-61		38		32		20		21
4-25-61		160		55		30		21
4-26-61		86		32		16		4
4-28-61		62		29		26		34
4-29-61		34		16		--		--
5-16-61		46		38		20		26
5-17-61		34		24		0		9
5-24-61		20		22		--		--
6-22-61		24		31		27		32
<u>Dog #17H</u>								
12-31-60				unilateral nephrectomy				
2- 8-61		26		38		22		22
4-11-61		20		26		42		28
4-12-61		4		29		8		30
5- 3-61		--		--		24		24
5- 6-61 a.m.		inoculated with 20 ml <u>Proteus</u> sp. via renal artery						
5- 6-61 p.m.		86		38		42		34
5- 7-61		34		29		26		26
5- 8-61		38		34		30		34
5-10-61		26		26		38		40

surgery involved. The other dog (18H) had an SGOT of 160 units the third day subsequent to inoculation into the renal artery. The following day the SGOT had dropped to 86 units and had returned to a normal of 34 units by the sixth day following inoculation. This information indicated the limitation of the SGOT for the detection of this type of nephritis. This also demonstrated the day-to-day variation of the SGOT during the course of disease. The above information emphasized that the clinician or research worker should not rely upon a single determination.

Serum Glutamic Pyruvic Transaminase

The SGPT findings are summarized in Table 5.

Table 5. Summary of serum glutamic pyruvic transaminase (SGPT).

Group No.	No. : dogs	No. : determinations	\bar{x} ¹	$s\bar{x}$ ²	s ³	Range : in units
I Normal	15	38	24.0	1.657	10.21	6-42
II Normal	28	103	29.9	1.021	10.41	12-55
III Distemper	7	7	21.7	10.68	28.21	2-83
IV CNS ⁴	11	11	36.6	6.77	17.87	22-82
V Nephritis	14	103	29.4	4.08	9.49	8-68

- 1 Mean in units
- 2 Standard error in units
- 3 Standard deviation in units
- 4 Central nervous symptoms

The SGPT of the two normal groups had essentially the same relationship as did the SGOT. The normal range was considered to be 13.8 to 34.2 units for group I and 19.5 to 40.3 units were considered to be within the normal range. The ranges of these two groups of dogs were nearly alike. Again, the standard error was small and the standard deviation was slightly larger than ten units. Normal mean SGPT was higher than had been reported by Ray (1958) and Cornelius et al. (1959) (Table 1). The slightly different enzymatic test used in this study may account for this difference.

The results of SGPT in dogs with natural distemper infection (group III) indicated that this test had little value as a diagnostic aid. The standard error and the standard deviation were

large. The sizable standard error indicated that there was considerable variability within each dog while the large standard deviation denoted that there was considerable dog-to-dog variation. All but one dog of this group were within the normal SGPT range.

Dogs with central nervous system disturbances had SGPT findings essentially the same as group III. Four of the 11 dogs of this group had SGPT readings above the normal range.

SGPT findings in dogs with nephritis were considered to be within the normal range. There were some border line elevations, but they were not considered to be of value as an aid to diagnosis of this type of nephritis.

Urine Glutamic Oxalacetic Transaminase

The UGOT findings are summarized in Table 6.

Table 6. Summary of urine glutamic oxalacetic transaminase (UGOT).

Group No.	No. dogs	No. determinations	\bar{x}	$s_{\bar{x}}^2$	s^3	Range in units
I Normal	16	46	26.0	1.743	11.82	4-55
II Normal	28	78	20.4	1.551	13.70	0-58
III Distemper	6	6	54.6	35.637	87.29	6-232
IV CNS ⁴	10	10	47.1	12.055	38.12	20-150
V Nephritis	14	81	25.2	7.432	13.80	3-83

1 Mean in units

2 Standard error in units

3 Standard deviation in units

4 Central nervous symptoms

The results of this work were contrary to the findings of Dunn et al. (1958) who found little or no transaminase activity in the urine of dogs. Every dog had significant transaminase levels in the urine at some time. The mean UGOT of group I was higher than that of group II. The normal range of UGOT of group I was 14.2 to 37.8 units and 6.7 to 34.1 units in group II.

Group III dogs did have a significantly elevated mean UGOT; however, only one dog of this group was above the normal range. This was also the case in group IV.

The mean UGOT of group V was considered normal.

Urine Glutamic Pyruvic Transaminase

Results of urine glutamic pyruvic transaminase (UGPT) findings are summarized in Table 7.

Table 7. Summary of urine glutamic pyruvic transaminase (UGPT).

Group No.	No. : dogs	No. :determi- : nations	\bar{x} ¹	$s_{\bar{x}}$ ²	s ³	Range : in : units
I Normal	16	46	27.2	1.114	8.55	0-50
II Normal	26	78	21.7	1.176	10.58	0-50
III Distemper	6	6	28.5	4.072	9.97	12-39
IV CNS ⁴	10	10	29.8	3.498	11.06	16-54
V Nephritis	14	80	25.0	4.779	8.70	7-40

- 1 Mean in units
- 2 Standard error in units
- 3 Standard deviation in units
- 4 Central nervous symptoms

The normal UGPT range was from 11.2 to 35.7 units.

All dogs in the other groups had UGPT readings that were considered to be essentially normal. The standard error was much larger in groups III, IV, and V. This indicated that there was a greater variability within the diseased dogs of the latter groups than there was within the normal dogs of groups I and II. The dog-to-dog variability remained relatively constant in all groups.

Cerebrospinal Fluid Glutamic Oxalacetic Transaminase

The findings of cerebrospinal fluid glutamic oxalacetic transaminase (CFGOT) are summarized in Table 8.

Table 8. Summary of cerebrospinal fluid glutamic oxalacetic transaminase (CFGOT).

Group No.	No. dogs	No. determinations	\bar{x}	s	s	Range in units
I and II ⁴						
Normal	43	43	20.0	1.62	10.62	6-46
III Distemper	8	8	41.8	18.004	50.92	4-150
IV CNS ⁵	14	14	81.5	18.245	68.24	20-260+

- 1 Mean in units
- 2 Standard error in units
- 3 Standard deviation in units
- 4 Groups I and II were combined in this analysis
- 5 Central nervous symptoms

The normal range of the CFGOT was from 9.4 to 30.6 units in these 43 dogs. The mean CFGOT was found to be approximately two-thirds the normal SGOT. This difference between normal CFGOT and

SGOT was not as great as the 50 per cent reported by Wakim and Fleisher (1956). The necessity for general anesthesia for collection of specimens limited the number of samples in the cerebrospinal fluid studies.

In group III the mean CFGOT was significantly higher than the normal. Three of the eight dogs had CFGOT readings of 150, 86, and 36 units. All others of this group were within the normal range. The extreme dog-to-dog variation was indicated by the large standard error of 50.92 units. This indicated that this test would be of limited value as an aid to the diagnosis of canine distemper.

Eleven of the 14 dogs in group IV had CFGOT readings above the normal range. This CFGOT elevation was probably related to the amount of cellular destruction of nervous tissue. These findings pointed out the possibility of using this test as an aid in the detection of central nervous system diseases. The limitations of this test were indicated by the large standard error and standard deviation.

Cerebrospinal Fluid Glutamic Pyruvic Transaminase

The cerebrospinal fluid glutamic pyruvic transaminase (CFGPT) results are summarized in Table 9.

The normal range of CFGPT was 7.5 to 21.1 units. The mean CFGPT was 6.6 units smaller than the corresponding CFGOT. This was the same general relationship that was found between the normal mean SGOT and SGPT.

Table 9. Summary of cerebrospinal fluid glutamic pyruvic transaminase (CFGPT).

Group No.	No. dogs	No. determinations	\bar{x} ¹	$s_{\bar{x}}$ ²	s ³	Range in units
I and II ⁴						
Normal	43	43	13.4	1.173	7.697	2-32
III Distemper	8	8	15.5	4.127	11.674	0-33
IV CNS ⁵	14	14	30.7	2.492	9.326	14-44

¹ Mean in units

² Standard error in units

³ Standard deviation in units

⁴ Groups I and II were combined for this analysis

⁵ Central nervous symptoms

There was only a slight difference in the CFGPT of group III and the normal. Only two dogs had CFGPT readings above the normal range with readings of 33 and 26 units.

As was the case with CFGOT, the CFGPT seemed to be generally elevated in group IV. Eleven of the 14 dogs in this group also had enzymatic elevations. However, these were not necessarily the same 11 dogs of this group that exhibited CFGOT elevations (Table 10).

By using a combination of the two (CFGOT and CFGPT), all dogs of this group except number 37 had either an elevated CFGOT or an elevated CFGPT, or both. This strongly indicated the need for utilizing both enzymatic determinations to study central nervous system diseases.

In considering the overall transaminase relationship, it was noticed that the spread between the mean SGOT and UGOT of group I

Table 10. Transaminase of dogs in group IV.

Dog No.	Units					
	SGOT	SGPT	UGOT	UGPT	CFGOT	CFGPT
6	70	32	26	24	160	40
13	162	32	54	34	--	--
20	150	38	--	--	162	24
24	138	50	54	40	>260	44
30	--	--	--	--	95	32
31	34	18	20	19	30	24
33	--	--	--	--	38	21
34	--	--	26	26	42	26
35	26	24	23	16	55	34
36	--	--	38	32	--	--
37	30	26	38	29	20	14
41	162	45	--	--	70	32
42	30	34	--	--	86	21
44	34	22	42	24	55	44
48	--	--	150	54	30	34
49	22	82	--	--	39	40

dogs was only 0.6 unit. The spread between the mean SGOT and UGOT of group II was 9.7 units. The same general relationship occurred in the case of SGPT and UGPT. No explanation could be given for this peculiar analogy. A speculative cause may have been the unilateral nephrectomy of the group II dogs.

There have been many different methods of determination devised (Karmen et al., 1955; Karmen, 1955; Tonhazy, 1950; Cabaud et al., 1956; Steinberg et al., 1956; Reitman and Frankel, 1957; Bowers et al., 1958; and King, 1958). After reviewing the literature and completion of this study, it was felt that a standard procedure should be adopted by the clinical pathology laboratories for determining the transaminases. Then a research worker could more accurately compare his results with those of another. The clinician could more accurately utilize the results of the

transaminase test. This was in agreement with Daly and Jordan (1959).

SUMMARY AND CONCLUSIONS

The normal mean SGOT was 26.6 units in group I dogs and 30.18 in group II dogs. The normal range SGOT was 17 to 36.2 units. The respective mean SGPT value of these two groups of dogs was 24.0 and 29.9 units. The normal range of SGPT for these dogs was 13.8 to 40.3 units. Dogs in these groups were considered to be normal.

The normal mean UGOT levels of groups I and II were 26.0 and 20.4 units, respectively. The normal range was from 6.7 to 34.1 units. These same groups of dogs had a normal UGPT range of 11.2 to 35.7 units. The mean UGPT for group I was 27.2 and the mean for group II and 21.7.

Normal mean CFGOT was 20 units with a normal range of 9.4 to 30.6 units. The normal CFGPT mean was 13.4 units with a normal range of 7.5 to 21.1 units.

Less spread was noticed between the SGOT and UGOT of dogs in group I than those of group II. Unilateral nephrectomy of the dogs in group II may have had some relationship to this phenomenon.

The transaminase tests were not significantly altered in the dogs with natural distemper infection. The transaminases of the dogs with experimental nephritis were not significantly altered although a transient rise was noticed in some of the dogs following the surgery associated with inoculation.

The CFGOT and/or CFGPT was elevated in 13 of the 14 dogs with central nervous system disturbance. This alteration was more consistent than transaminase from the urine or blood.

The standard error and standard deviation were generally greater in the diseased dogs than in the normal dogs. The adoption of a standardized transaminase procedure was recommended. The need for a series of determinations from a patient was indicated from these studies.

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TRANSAMINASE ACTIVITY IN SERUM, URINE, AND
CEREBROSPINAL FLUID OF NORMAL AND DISEASED DOGS

by

CLAIR M. HIBBS

B. S., University of Missouri, 1949
D. V. M., University of Missouri, 1953

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MASTER OF SCIENCE

Department of Pathology
School of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Transaminases have been used extensively in diagnostic and research work in the field of human medicine. This clinical pathological aid has more recently become prominent in the field of small animal veterinary medicine. Spectrophotometric transaminase analysis with prepared reagents has come into use while most of the normal enzymatic ranges were established by a slightly altered method. The transaminases in normal canine urine and cerebrospinal fluid has had limited study. The need for establishing the normal range of SGOT, SGPT, UGOT, UGPT, CFGOT, and CFGPT using prepared reagents was apparent. There was also a need of establishing the day-to-day variation of normal dogs and animal-to-animal variation of normal dogs. These normal variations can then be applied to diseased animals. Each normal urine transaminase was derived from 124 samples, and each normal serum transaminase was derived from 141 samples. The normal range SGOT was 17 to 36.2 units; the normal range SGPT was 13.8 to 40.3 units. The normal range UGOT was 6.7 to 34.1 units, and the normal range UGPT was 11.2 to 35.7 units.

There was a greater difference between SGOT and UGOT of normal dogs that had previously been unilaterally nephrectomized than the other group of normal dogs. Removal of one kidney may have had some bearing upon this difference.

One cerebrospinal fluid specimen was collected from each of 43 dogs. The normal range CFGOT was 9.4 to 30.6 units and the normal range CFGPT was 7.5 to 21.1 units.

Three groups of abnormal dogs were studied. One group of diseased dogs consisted of dogs inoculated intravenously with

Proteus sp. The dogs were inoculated by one of the following methods: (1) washed saline suspension of the organism via renal artery, (2) washed saline suspension of the organism via peripheral vein, or (3) 0.25 ml of Proteus sp. culture in tryptocase soy broth with 0.1 per cent agar added via the renal artery.

A transient elevation did occur as a result of surgery performed in the process of inoculating the dogs via the renal artery. The nephritis produced did not result in a significant elevation of the transaminases in urine or blood.

Terminal specimens of blood urine and cerebrospinal fluid were collected from seven dogs with a natural infection of canine distemper. The transaminases were not consistently significantly altered.

Sixteen dogs that had been inoculated intracerebrally with a canine distemper street virus and developed central nervous symptoms were studied.

Blood, urine, and cerebrospinal fluid was collected prior to euthenasia, and the amount of transaminase in the specimens was determined. The SGOT and SGPT were slightly elevated in 3 dogs. The CFGOT and/or CFGPT was elevated in 13 of the 14 cerebrospinal fluid samples utilized. The urine transaminase changes were not significant.

The standard error and standard deviation were greater in diseased dogs than in normal dogs. This study indicated the need for the study of a series of specimens rather than one single specimen. The adoption of a standard procedure for determining transaminases was recommended.