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Transaminases in Blood, Urine and Cerebrospinal Fluid of Normal and Unilaterally Nephrectomized Dogs.* (30045)

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Transaminases are intracellular enzymes normally found in body fluids as a result of the normal catabolic processes and/or pathologic processes. Quantitative determinations of these enzymes have been used extensively in human medicine to detect cellular destruction. The development of simplified laboratory procedures for transaminase determinations has stimulated increased use of such tests in the study of animal diseases.

Serum transaminase levels have been reported in dogs(2,6,9). However, a few reports concerning the levels of these enzymes in urine and cerebrospinal fluid of the canine have been published.

This study was undertaken in an attempt to establish and compare glutamic oxalacetic and pyruvic transaminase levels in blood, urine and cerebrospinal fluid of control and unilaterally nephrectomized dogs.

Mean serum glutamic oxalacetic transaminase (SGOT) of mongrel dogs was found to be 39 $\pm$ 3.5 units(6) using the method of LaDue, Wroblewski and Karmen(4,5). Other investigators reported a mean level of 22.7 $\pm$ 5.4 Sigma-Frankel units SGOT in dogs 9 months of age and mean SGOT level of 18.6 Sigma-Frankel units in the dog(2,10). Mean serum glutamic pyruvic transaminase (SGPT) levels of 21.8 $\pm$ 6.2 and 22.5 Sigma-Frankel units have been reported(2,10).

Presence of lysed red blood cells in serum resulted in greater glutamic oxalacetic transaminase (GOT) activity. However, significant changes were not found in transaminase levels when serum and plasma from heparinized and oxalated blood were studied. No significant changes were found in serum collected at various times during the day(14).

Urine samples from apparently normal humans were found to contain slight, insignificant amounts or less than 1 unit/ml GOT (1,8,15). Little or no transaminase activity has been found in dog urine. It was postulated that either the enzyme molecule was too large to pass through the glomerular filter or tubular cells were capable of completely reabsorbing the enzyme after filtration(3).

Normal cerebrospinal fluid glutamic oxalacetic transaminase (CFGOT) in infants was reported to be 1 to 10 units/ml with a mean of 4.5 $\pm$ 2.2 units(7). Cerebrospinal fluid glutamic oxalacetic transaminase level in dogs was 0.66 uM/ml/hr $\pm$ 0.08 using the method of Karmen(4,15).

Methods. Dogs of mixed breeds, both sexes, less than 2 years of age, and vaccinated against canine distemper were used. Group I dogs were non-nephrectomized controls. Group II consisted of dogs which had been subjected to surgical unilateral nephrectomy.

Tissue destruction due to surgery is reported to cause a transient elevation of SGOT (12, 13, 17). This elevation may occur within one to four days after surgery. To avoid this, samples were not collected from dogs in Group II for one month following nephrectomy. Tissue destruction associated with surgery was, therefore, not a factor in any alteration of transaminase levels in Group II dogs.

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TABLE I. Summary of Transaminase Studies.

Group No.	No. dogs	No. determinations	Kind determinations	Mean	S.E.	S.D.	Range
				In units			
I. Control	15	38	SGOT	26.6	1.55	9.58	12-55
II. Unilateral nephrectomy	28	103	"	30.2	1.13	11.50	4-66
I. Control	15	38	SGPT	24.0	1.65	10.21	6-42
II. Unilateral nephrectomy	28	103	"	29.9	1.02	10.41	12-55
I. Control	16	46	UGOT	26.0	1.74	11.82	4-55
II. Unilateral nephrectomy	28	78	"	20.4	1.55	13.70	0-58
I. Control	16	46	UGPT	27.2	1.11	8.55	0-50
II. Unilateral nephrectomy	26	78	"	21.7	1.18	10.58	0-50
I. Control	37	37	CFGOT	20.1	1.64	10.08	9-46
II. Unilateral nephrectomy	10	10	"	24.8	3.88	12.28	6-42
I. Control	37	37	CFGPT	13.7	1.35	8.26	2-32
II. Unilateral nephrectomy	10	10	"	14.8	2.12	6.71	6-30

Blood samples were collected by venous puncture. Only non-hemolyzed samples were utilized. Urine was collected by catheterization or urinary bladder tap. Cerebrospinal fluid was collected by flexing the head of an anesthetized dog ventrally and locating the area just dorsal to the foramen magnum. The area was scrubbed with a disinfectant. A sterile 20-gauge, one-inch needle attached to a sterile 5 ml glass syringe was inserted into the foramen magnum. Two ml of cerebrospinal fluid was aspirated. Only clear samples were saved for testing.

Specimens were stored at —20°C or used the day collected. Refrigerated samples were allowed to warm to room temperature prior to testing. No sample was stored more than 2 weeks.

Transaminase activity was determined according to modified method of Reitman and Frankel(11) as outlined in the reagent manufacturer's directions.† The optical density was recorded at 505 mμ, using a Coleman Jr. Spectrophotometer,‡ and the units of GOT or GPT activity determined by use of a calibration curve. Urine pigments did not affect optical density.

Results. All enzymatic determinations are summarized in Table I. The mean SGOT was 26.6 ± 1.55 units in Group I and 30.2 ± 1.13 units in Group II. The SGOT range in Group I dogs was 12 to 55 units. By comparison, the range in Group II was 4 to 66 units. The dog to dog SGOT variation was 9.58 units in Group I and 11.5 units in Group II. The mean difference was considered normal for this method of determination and standard errors in these groups were small.

Essentially the same relationship existed in the SGPT studies. Group I had a mean SGPT of 24.0 ± 1.65 units while the mean SGPT of Group II was 29.9 ± 1.02 units. The SGPT range was 6 to 42 units in Group I and 12 to 55 units in Group II. The standard error was small, and the standard deviation was just over 10 units in both groups.

The mean urine glutamic oxalacetic transaminase (UGOT) of Group I was 26.0 ± 1.74 units while that of Group II was 20.4 ± 1.55 units. The range UGOT of Group I was 4 to 55 units and 0 to 58 units in Group II. The standard deviation was 11.82 units in Group I and 13.70 units in Group II.

The mean urine glutamic pyruvic transaminase (UGPT) was 27.2 ± 1.11 units in Group I and 21.7 ± 1.18 units in Group II. The UGPT range in Group I was 0 to 50 units with an identical range in Group II. The standard deviation of Group I was 8.55 units while in Group II it was approximately 2 units higher.

The mean CFGOT was 20.1 ± 1.64 and 24.8 ± 3.88 units for Groups I and II respectively. The range of Group I CFGOT was 9 to 46 units and 6 to 42 units in Group II. The standard deviations were 10.08 and 12.28 units for Group I and II respectively.

† All reagents for GOT and GPT determinations were supplied by Dade Reagents, Inc., Miami, Fla.

‡ Coleman Instruments Inc., Maywood, Ill.

The cerebrospinal fluid glutamic pyruvic transaminase (CFGPT) range of Group I was 2 to 32 units and 6 to 30 units in Group II. The mean CFGPT of Groups I and II was 13.7 ± 1.35 and 14.8 ± 2.12 units respectively. The standard deviation was 8.26 units in Group I and 6.71 units in Group II.

Discussion. Using the confidence interval, there was a statistically significant difference between Group I and Group II mean SGOT. However, using the same method of statistical analysis there was also a significant difference between 2 separate studies of apparently normal dogs(2,10). These workers used the same enzymatic method. The mean SGOT of Group I in this study was 8.0 units and 3.9 units higher than was found by others (2,10) but 12.9 units lower than was found in earlier studies(6). This variation is probably due to difference in care, feed, age and other variables. The standard error of the SGOT from Group I was 1.55 units and 1.13 units for Group II. The standard errors of SGOT studies in apparently normal dogs has been reported as being 5.4(2) units and 3.5 units(6). The test used was relatively accurate when compared to other studies.

The results of UGOT determinations do not correlate with previously reported levels of either no detectable GOT or less than one unit of transaminase activity(1,3,15). Each animal had a detectable amount of GOT in its urine at some time during the study. Occasionally, however, a single urine sample did not contain demonstrable UGPT and/or UGOT. It is interesting to note that the range of UGPT was the same in both groups, but the mean UGPT level of Group I was 5.5 units larger than Group II.

Unilateral nephrectomy tended to decrease the quantity of transaminases eliminated *via* the urinary system. Dogs with one kidney removed had a higher blood level of both GOT and GPT than control dogs. Thus, it would appear that the kidney had some relationship to elimination, alteration and/or destruction of transaminases.

The CFGOT of the control dogs was 6.5 units less than the SGOT. The spread between GOT levels of these 2 body fluids was less than the 50% difference previously reported(16). The CFGOT of the unilaterally nephrectomized group was 5.4 units less than the SGOT.

The mean CFGOT level of Group I was 4.7 units less than that of Group II. Since the standard error of Group II was more than double that of Group I, it appeared that unilateral nephrectomy had little effect on the level of this enzyme in cerebrospinal fluid. The wide ranges of both groups produced information which further substantiated the fact that unilateral nephrectomy had little or no effect upon the CFGOT level.

Results of the CFGPT studies in these 2 groups of dogs did not indicate that unilateral nephrectomy had any significant effect upon the level of this enzyme in the cerebrospinal fluid. The difference between the CFGPT means of these 2 groups was only 1.1 unit, Group II being the larger.

Summary. Transaminase studies were made of control (Group I) and unilaterally nephrectomized (Group II) dogs. Transaminase determinations were made of serum, urine and cerebrospinal fluid of both groups (Table I). There was increased SGOT and SGPT level but a decreased UGOT and UGPT level as a result of unilateral nephrectomy. Removal of one kidney had little effect upon the transaminase enzyme level in the cerebrospinal fluid.

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Protection Induced by Bacterial Endotoxin Against Whole-Body X-Irradiation in Germfree and Conventional Mice.* (30046)

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A number of naturally produced microbial substances including dextran(1,2), levan(3), zymosan(3,4), bacterial endotoxins(4,5), and Gram-positive antigens(5) have been tested for their ability to protect mammals exposed to whole-body ionizing radiation. The best known of these are the bacterial endotoxins derived from Gram-negative enteric bacteria, and chemically defined as high molecular weight complex polysaccharides(6) which have provided some measure of protection from irradiation in several rodent species(5). Protection measured as increased survival is dependent on time and dose of endotoxin inoculation in relation to time of irradiation, as well as dose and type of radiation(5,7,8).

Recent studies have shown that the composition of the intestinal flora plays a major role in ability of mice to respond to bacterial endotoxins(9,10). Pathogen-free as well as specific pathogen-free (SPF), and germfree mice have been shown to be more resistant to the lethal effects of *Escherichia coli* endotoxin than conventional mice(10,11). Monoinfection with coliform bacteria does not induce susceptibility to endotoxin in ex-germfree animals(12). Prior contact with heat-killed or living bacteria rendered Rockefeller Swiss (NCS) albino mice more susceptible to the lethal effects of endotoxin, especially when that endotoxin was prepared from the infecting bacterial strain(11). Physiological reactions to small doses of endotoxin were essentially prevented in NCS mice previously vaccinated with dead bacteria.

Other evidence indicates that germfree and conventional mice are equally susceptible to the toxic effects of endotoxin from *Salmonella enteritidis* and *S. flexneri*(13). Certain immunological responses to small doses of these endotoxins were found to be similar in both groups of animals. On this basis, there is little difference in type or magnitude of reaction to bacterial endotoxin in animals in constant contact with living Gram-negative bacteria and those denied such contact.

Since the dose of bacterial endotoxin required for radiation protection is small, its effects may be altered according to the number and species of endogenous bacteria liberating endotoxin in the intestinal tract. Conceivably, such an animal may have the capability of immunological neutralization of small amounts of endotoxin rendering that dose partially or totally ineffective. By the same reasoning, germfree mice may show exaggerated responses to endotoxin resulting in a greater degree of radiation protection. To obviate the interference of endotoxin produced by endogenous bacteria and to discern to what degree the presence of Gram-negative enteric bacteria alter these responses, germfree animals are the experimental animals of choice. We report here the results of a comparative study of the alterations in the dose-response curves of conventional and germfree mice exposed to X-irradiation 24 hours after receiving 10 μg of endotoxin.

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