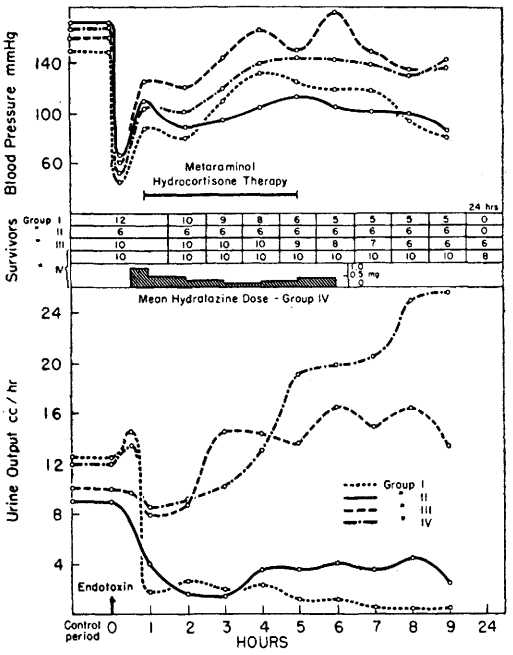


FIG. 1. Time-dose relationship of 4 groups of dogs given a lethal dose of endotoxin. Group 1 = untreated controls; group 2 = treated with hydralazine alone; group 3 = treated with hydrocortisone and metaraminol; group 4 = treated with hydrocortisone, metaraminol and hydralazine. Curves illustrate avg hourly blood pressure and output of urine. Animals surviving 24 hr after endotoxin are indicated for each group.

Discussion. In studies on the pathogenesis of canine endotoxin shock attempts have been made to reverse the peripheral vascular collapse with various agents. After onset of hypotension, and oliguria or anuria, the most consistently successful results obtained in this laboratory have followed administration of a combination of hydrocortisone and metaraminol. The present study has demonstrated that hydralazine will increase urine flow in dogs having severe hypotension. This is in contrast to the untreated control series of animals that had a higher blood pressure but a consistently lower output of urine. When the systemic pressure was supported with a pressor drug and steroid, hydralazine had a favorable effect on output of urine, even though the blood pressure was lower than in the pressor-steroid treated group. These results are of interest since hydralazine is usually employed as an anti-hypertensive drug.

Summary. Canine endotoxin shock is characterized in part by hypotension, oliguria, hemoconcentration and acidosis. The "shock" can be reversed in some animals with a combination of a pressor drug and hydrocortisone. The addition of hydralazine to this combination greatly augments the flow of urine, and possibly contributes to the survival of animals.

1. Spink, W. W., Vick, J., *Circulat. Res.,* 1961, v4, 184.

2. Hinshaw, L. B., Bradley, G. M., Carlson, C. H., *Am. J. Physiol.,* 1959, v196, 1127.

3. Reubi, F., *Helvet. Med. Acta,* 1949, v16, 297.

4. Freis, E. D., Rose, J. C., Higgins, T. F., Finnerty, F. A., Jr., Kelly, R. T., Portenope, E. A., *Circulation,* 1953, v8, 199.

5. Grob, D., McKusick, V. A., *Johns Hopkins Hosp. Bull.,* 1955, v96, 164.

6. Judson, W. E., Hollander, W., Wilkins, R., *Circulation,* 1956, v13, 664.

7. Weil, M. H., MacLean, L. D., Visscher, M. B., Spink, W. W., *J. Clin. Invest.,* 1956, v35, 1191.

Received October 31, 1960. P.S.E.B.M., 1961, v106.

Presence of Two Glutamic-Oxalacetic Transaminases in Serum of Dogs Following Acute Injury of the Liver.* (26310)

GERARD A. FLEISHER AND KHALIL G. WAKIM
(With technical assistance of Marcella Pankow, David Osborne and Lester Clapp)
Sections of Biochemistry and Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minn.

We have reported recently that human and animal tissues such as heart and liver contain 2 glutamic-oxalacetic transaminases

* This investigation was supported in part by research grant from Nat. Inst. of Arthritis and Metab. Dis., P.H.S.

(1). At pH 7 the previously known enzyme (GOT I) has anionic properties, whereas the newly discovered transaminase (GOT II) acts as a cation. Separation, therefore, can be made easily by electrophoresis or by use of ion exchangers(2). The relative proportion of the 2 enzymes also can be determined indirectly by a differential test. In most tissues investigated the contribution of GOT II to total activity has been found to vary between 40 and 70%. In normal human serum the presence of GOT II has not been established with certainty, and in a few serums with high transaminase activity due either to recent myocardial infarction or to acute viral hepatitis the proportion of GOT II was found to be small. It was thought to be of interest to determine in dogs acutely poisoned with carbon tetrachloride whether the high serum activity of GOT resulting from this injury(3) might contain at any time substantial proportions of the new transaminase. Because activity of enzymes in serum must also depend on rate of elimination, we have studied in dogs the disappearance curves of intravenously injected, highly purified preparations of the 2 GOT's as well as of GPT (glutamic-pyruvic transaminase).

Methods. Production of hepatic injury. Four healthy dogs received carbon tetrachloride by gastric intubation in doses of 2 ml per kilo of body weight. Blood specimens were taken from the external jugular vein, irrespective of food intake, twice or three times before the beginning of the experiment, to serve as controls, and over a period of 12 days following administration of the toxic agent. The blood was allowed to clot and the serum was separated by centrifugation.

Determination of enzymatic activities. Determination of transaminase activities was by the differential spectrophotometric test at 38°C, as previously described(1). Activities are expressed as micromoles of oxalacetic acid formed per hour per milliliter of serum (μM/hr/ml). The differential test consists of 2 determinations, one at pH 7.4 in presence of high concentration of aspartate (10^{-1} moles/liter) to determine the sum of activities of GOT I and GOT II, the other at pH 6.0 in presence of low concentration of aspartate (5×10^{-3} moles/liter) to determine almost exclusively GOT II. Corrections for the slight nonenzymatic destruction of DPNH (reduced diphosphopyridine nucleotide) were applied in each case; in our experience these amount to 1.5 and 2.2 density units per minute at pH 7.4 and 6.0, respectively. Activity quotients (activity at pH 6.0 divided by activity at pH 7.4) for our purest preparations from dog liver were 0.544 and 0.027, respectively, for GOT II and GOT I. From the activity quotient determined in a serum with an unknown mixture of the 2 transaminases the proportion of each activity can be calculated with a fair degree of accuracy.

Preparation of purified transaminases and intravenous administration to dogs. The method of separation and purification of the 3 transaminases, GOT I, GOT II and GPT, from dog liver was presented by one of us (2) at a recent meeting and will be the subject of a forthcoming paper. The preparations used for intravenous injection were purified several hundredfold. Specific activities based on milligram protein nitrogen were 35,000, 60,000 and 20,000, respectively, for GOT I, GOT II and GPT. The preparations were injected in doses of about 15,000 μM/hr per kilo of body weight, contained in 0.3 ml of solution. Serial blood specimens were taken until serum activity had returned to normal values.

Results and discussion. Carbon tetrachloride poisoning. In confirmation of earlier tests(3), maximal total GOT activity was observed in all 4 dogs 48 hours after administration of carbon tetrachloride. Activities then ranged from 448 to 995 μM/hr/ml as compared with 0.89 to 1.40 for control specimens. By the ninth day values had returned almost to normal. The findings in one dog are summarized in the Table, while individual activities of GOT I and GOT II, calculated from the activity quotients, for the 4 dogs, are plotted in Fig. 1. For the periods between 16 and 96 hours it is seen that at the time of maximal total activity both enzymes exhibited peaks. Activity quotients then ranged from 0.10 to 0.11, an indication that GOT II contributed 14 to 16% (mean

TABLE I. Distribution of Serum Glutamic-Oxalacetic Transaminases Following Administration of Carbon Tetrachloride in a Dog.

Hr after CCl_4	GOT, μM/hr/ml pH 7.4	pH 6.0	Quotient	GOT II %	μM/hr /ml
Control*	1.04	.052	.050		0
"	1.22	.030	.025		
"	1.33	.017	.013		
16	44.0	1.13	.026	0	0
18	41.6	1.15	.028	.2	.1
20	43.2	1.85	.043	3.1	1.3
24	54.0	2.19	.041	2.7	1.4
40	760.0	68.3	.090	12.2	92.8
48	824.0	82.4	.100	14.1	116.2
64	139.4	7.1	.051	4.7	6.6
72	47.5	3.06	.064	7.2	3.4
88	11.3	.74	.065	7.6	.9
96	8.5	.62	.073	8.9	.8
112	6.5	.37	.057	5.8	.4
168	3.9	.23	.059	6.4	.2
192	3.3	.19	.058	6.0	.2
216	1.6				
240	2.2				
264	1.8				
288	1.4				

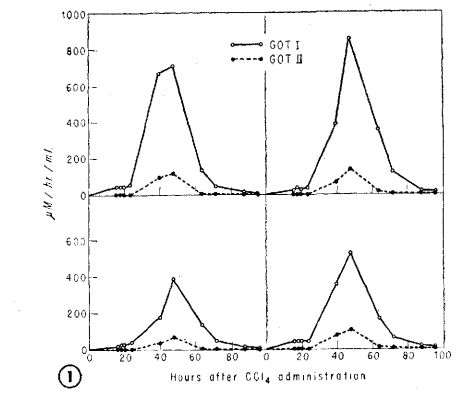
* 4, 2 and 0 days before inj. of CCl_4.

14.7) to total activity. At 40 hours the distribution was quite similar, while at 24 and 64 hours, GOT II averaged 3.5 and 4.5%, respectively, of total activity. Whether, in normal dog serum, GOT II is present at all is difficult to decide, since its activity at pH 6.0 is extremely small and the accuracy of the test, therefore, low. Mean quotient with standard error of the mean in 9 control serums was 0.029 ± 0.006, which is nearly the same as the quotient of purified GOT I (0.027). Interestingly enough, in rat serum we have found that GOT II contributes 20 to 30% to total activity.

It may be of interest to recall the findings on GPT following administration of carbon tetrachloride to dogs(3). Peak activity generally occurred 1 day later than did that of GOT, and was also somewhat higher. GPT activity also decreased more slowly than did that of GOT, with the result that the activity-time curve of GPT was very much broader than that of GOT. On the thirteenth day after administration of the hepatotoxic agent, GPT activity was still 16 to 20 times control values.

Intravenous administration of transaminases. Disappearance rates of exogenous GOT I, GOT II and GPT in normal dogs are shown in Fig. 2. The 3 enzymatic preparations were also injected into groups of 3 additional dogs and the decline of their activities followed. In each instance the results were quite similar to the ones illustrated. GOT II disappeared very rapidly from the blood stream, and 6 to 8 hours after injection usually none of the injected enzymatic activity remained. A much slower elimination was the rule for GOT I. Here enzymatic activity did not return to control levels until 3 to 4 days after injection. The slowest rates were observed for injected GPT, which was present in blood for as long as 18 days.

It seems that the differences in elimination curves of exogenous GOT I, GOT II and GPT bear a relationship to the activity curves in serum of endogenous transaminases following poisoning of the liver by carbon tetrachloride. GOT II is eliminated so rapidly that it cannot build up as high a concentration in blood as can GOT I or GPT. GPT, on the other hand, disappears so slowly that it shows a numerically greater maximal



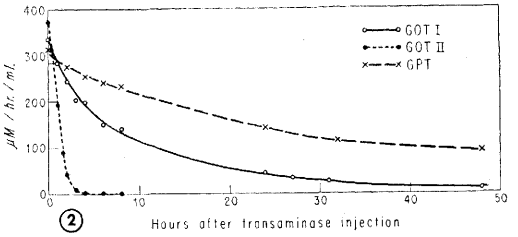


FIG. 1. Serum activities of GOT I and GOT II following carbon tetrachloride poisoning in 4 dogs.
FIG. 2. Serum transaminase activities following intrav. administration of purified transaminase preparations in 3 dogs.

activity than does GOT I and remains elevated for a considerably longer time after hepatic injury. Similar observations with regard to (total) GOT and GPT also have been made in patients with acute viral hepatitis or jaundice due to chlorpromazine(4-8), but a detailed study of the distribution of GOT I and GOT II in these conditions has not been made.

Another reason for the very low levels of serum GOT II in carbon tetrachloride poisoning may be sought in the recently discovered fact(9) that this enzyme is bound within the mitochondrion, in contrast to GOT I and GPT which appear to be present exclusively in the soluble part of the cell. While GOT II is readily released from its confinement inside the mitochondrion in the preparation of aqueous homogenates, we have noted that the enzyme is quite tightly bound in mitochondria isolated by standard procedure, and is then difficult to solubilize. It is conceivable that also under physiologic and pathologic conditions this enzyme may be released with some difficulty.

Summary. Serums of dogs poisoned with carbon tetrachloride exhibited high activities of GOT I and GOT II with maxima 48 hours after administration of the toxic agent. At no time, however, did GOT II contribute to total activity more than 16%. In control serums the presence of GOT II could not be established with certainty. Disappearance rates of intravenously administered purified preparations of transaminase also were studied in dogs. Activity of GOT II decreased very much faster than that of GOT I. This difference may explain the uneven distribution of the 2 serum transaminases in carbon tetrachloride poisoning.

1. Fleisher, G. A., Potter, C. S., Wakim, K. G., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 229.

2. Fleisher, G. A., *Fed. Proc.,* 1960, v19, 6.

3. Fleisher, G. A., Wakim, K. G., *Proc. Staff Meet., Mayo Clin.,* 1956, v31, 640.

4. DeRitis, Fernando, Coltorti, Mario, Giusti, Giuseppe, *Clin. Chim. Acta,* 1957, v2, 70.

5. Chinsky, Murray, Wolff, R. J., Sherry, Sol, *Am. J. Med. Sci.,* 1957, v233, 400.

6. Wróblewski, Felix, *A. M. A. Arch. Int. Med.,* 1957, v100, 635.

7. Pryse-Davies, John, Wilkinson, J. H., *Lancet,* 1958, v1, 1249.

8. Sommerville, R. L., Fleisher, G. A., Dearing, W. H., *Gastroenterology,* 1960, v39, 574.

9. Rosenthal, Otto, Thind, S. K., Conger, N., *Am. Chem. Soc. Meeting,* New York, Sept. 1960, 10c.

Received November 7, 1960. P.S.E.B.M., 1961, v106.

Glycogen Content of Normal Lymphocytes.* (26311)

SANFORD L. LEIKIN (With technical assistance of Robert H. Carter)
(Introduced by Samuel P. Bessman)
Research Foundation, Children's Hospital of the District of Columbia, Washington

The glycogen content of the formed elements of the blood is said to reside in the leukocytes, red cells and platelets containing little or none of this polysaccharide(1). Furthermore, it has been assumed that the major portion of that present in the white blood cells is contained in the myeloid elements. These assumptions are based on histochemical examination of the blood from normal individuals and leukemic patients(2,3) and from chemical determinations on the leukocytes from patients with acute and chronic lymphocytic leukemia(4,5). Glycogen assays of the lymphocytes found in these states have revealed extremely low levels. It has therefore been deduced that the lymphocyte of the normal individual contains a meager amount of this polysaccharide. However, direct measurements of normal lymphocytes have not been possible because of the difficulty of separating these cells from the other formed elements of the blood except when they are present in elevated and ex-

* Supported by a grant from the Margaret and Alexander Stewart Fund.