Serum Arginase Activity.* (23173)

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Assays for various serum enzymes, such as transaminase(1) and aldolase(2) have been utilized to detect hepatocellular necrosis or damage. These enzymes, however, are present in all tissues, and gross alterations in their serum activities are not specific for liver damage. Since arginase is present in significant quantity only in liver(3), we have explored the usefulness of measuring activity of this enzyme in normal sera and in the sera of patients with liver disease.

Method. Blood samples are collected in unoxalated tubes and the serum separated and frozen until assay. One part of serum (usually 2 ml) is mixed with 2 parts saturated ammonium sulfate (pH 9) and centrifuged at 4000 x g for 15 minutes at 0°C. The supernatant fluid is discarded. The protein precipitate is taken up in saline and the ammonium sulfate precipitation repeated. This procedure removes the bulk of the urea present in the serum. The assay for arginase is carried out routinely by incubating in centrifuge tubes at 38°C for 30 minutes the twice ammonium sulfate precipitated proteins of the serum (usually the equivalent of 1 ml of serum) with the following components in a final volume of 2 ml: 0.5 ml of 0.2 M maleate buffer at pH 7.0(4) (containing 0.05 M $MnCl_2$) and 0.5 ml of 1.14 M L-arginine, adjusted to pH 9.4. The pH of the final mixture is 9.0. A blank without serum is included as well as blanks without arginine for the estimation of endogenous urea. Activity is proportional to enzyme concentration provided that no more than 1% of the substrate has been utilized(4). The reaction mixture is deproteinized by the addition of 2 ml of 1 M $HClO_4$. The tubes are centrifuged at 2000 x g for 10 minutes and aliquots of the protein-free supernatant fluid are analyzed for urea by the method of Archibald(5) with the following modifications: Aliquots containing no more than 1 μmole of urea (usually 2 ml) are made up to 5 ml and 2 ml of a 3 to 1 mixture H_2SO_4-H_3PO_4 mixture (technical H_2SO_4 should be used—presumably due to the requirement for trace metals(6,7)) and 0.4 ml of isonitrosopropriophenone (4% in ethanol) are added. The contents are mixed, the tubes capped with rubber stoppers fitted with capillary tubes, and heated in a covered, boiling water bath for one hour. After cooling in the dark (the color is light sensitive and should be protected from direct sunlight), a reading at 540 mμ is obtained. Since color development deviates from Beer's Law, a calibration curve is necessary.

Results. Table I records the mean and standard deviation of the arginase activity obtained in sera of 34 healthy blood donors. Table II records the values obtained in patients with liver damage. Values are recorded in μmoles of urea per ml serum per 30 minutes incubation at 38°C.

Discussion. Removal of the urea normally present in the serum by ammonium sulfate precipitation is not complete but the residuum is small. That control tubes should be run is emphasized by inspection of the experiment with serum from a patient with high blood urea level (Case No. 17; blood urea 210 mg %) which would have given erroneous results without control tubes. It is apparent from the tables that there is considerable variation in the blank values. Whether this is due to intrinsic variations in these

TABLE I. Serum Arginase Determinations in 34 Healthy Blood Donors.

♂	♀	Mean age	μM urea: Control tube	μM urea: Assay tube	2 – 1
29	5	28.5	.10 ± .07	.14 ± .16	.06 ± .09

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TABLE II. Serum Arginase Determinations in 19 Patients with Liver Disease.

No.	Age	Sex	μM urea Control tube	μM urea Assay tube	2 – 1	Diagnosis
1	19	♂	.00	.72	.72	Infectious hepatitis
2	40	♂	.00	1.98	1.98	Laennec's cirrhosis§
3	40	♂	.18	1.02	.84	*Idem* §
4	52	♂	.20	.89	.69	"
5	35	♀	.13	.19	.06	? "
6	35*	♀	.00	1.20	1.20	" §
7	35†	♀	.00	1.36	1.36	" §
8	47	♀	.03	.39	.36	"
9	38	♀	.42	1.56	1.14	"
(10)	33	♀	.18	.02	.00	? "
11	19‡	♂	.24	.10	.00	Serum hepatitis‡
12	50	♀	.12	.65	.53	Post-necrotic cirrhosis
13	50	♀	.06	2.38	2.32	*Idem*
14	45	♀	.39	6.47	6.08	Cardiac cirrhosis
15	45	♀	.45	5.95	5.50	*Idem*
16	45	♀	.42	3.08	2.66	"
(17)	61	♂	.85	1.09	.24	Hepatoma§
18	48	♂	.28	.43	.15	Fatty infiltration
19	38	♀	.24	.20	.00	Hepatic coma (etiology ?)
	Mean		.22 ± .23	1.56	1.36	

* Simultaneous peripheral vein sample. † Hepatic vein wedge sample. ‡ Recovery phase. § Confirmed by autopsy or biopsy.

samples, as for example higher endogenous urea or to other chromogenic materials which are occluded during the ammonium sulfate precipitation, is not known.

Case No. 10 represents a patient with clinically suspected liver disease which was not confirmed by laboratory studies. The arginase activity was zero in this case.

Assay of ornithine formation has not proved technically feasible for routine assay.

Summary. Serum arginase activity has been determined in 34 healthy blood donors. A mean value 0.06 ± 0.09 μmole urea formed/ml serum was found with a range of .00 to 0.36 μmole. In sera of 19 patients with proven or suspected liver disease the mean arginase activity was 1.36 with a range of .00 to 6.08 μmoles formed/ml serum under the condition of assay.

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