Serum Enzyme Studies of a Hydrocephalic Syndrome of Newborn Calves.* (27906)

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Various serum enzymes are significantly elevated in a variety of pathological conditions associated with destruction of tissues. Elevated serum glutamic oxaloacetic transaminase (GOT) has been reported in progressive muscular dystrophy in man(1,2,3). Chowdhury *et al.*(3) reported that patients with rapidly progressive (acute) muscular dystrophy had high serum levels of malic dehydrogenase (MDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH), and aldolase. However, patients with slowly progressive (chronic) dystrophy had normal serum enzyme levels except for aldolase which was slightly elevated. In acute human myocardial infarction the serum GOT rose within 12 to 24 hours and returned to the normal range within 3 to 6 days(4). However, serum GOT activity in patients with acute febrile and chronic infectious disease were found in the normal range(4). Experimental muscular atrophy induced by neurotomy, cauda equina section, tenotomy or vascular constriction did not appear to affect the level of serum GOT(5). Elevated serum transaminase levels in lambs and calves affected with white muscle disease have been reported(5,6,7). Elevation in serum GOT has been observed in ewes given multiple doses of CCl_4. However, there was no appreciable elevation in GPT(8,9). The rapid increase in serum GOT, GPT, LDH, MDH, and aldolase has been interpreted as due to the breakdown of tissues rich in these enzymes which are then released into the blood (1,3,4). Blincoe and Dye(6) found that in calves and sheep with while muscle disease, the increase in serum GOT appeared to be proportional to muscle damage.

A recent study has shown that a hereditary hydrocephalic syndrome in newborn calves was in fact an "encephalo-ophthalmomyopathy"(10). The progressive muscular dystrophy observed morphologically has stimulated investigation into the serum enzymes in these calves and the present report concerns the study of serum enzyme levels of MDH, LDH, GOT, GPT, and aldolase in this hereditary disease.

Methods. Serum was obtained from 3 Hereford and one Ayrshire calves which showed the hydrocephalic syndrome. The calves were approximately one day old. The serum was frozen until all samples were collected. Serum samples obtained from 6 normal calves ranging in age from one to 6 days were used as controls. Enzymatic analyses were run on all of the sera concurrently.

MDH and LDH activities were determined according to the method of Mehler *et al.*(11). These assays were performed with the use of a Beckman Model DB spectrophotometer connected to a Sargent's Model SR recorder. GOT and GPT were performed according to the procedure outlines by Sigma Technical Bulletin No. 505(12). Aldolase analyses were performed according to Sibley and Lehninger(13) and Sigma Tentative Technical Bulletin No. 750(14).

Total protein concentrations were measured by the method of Lowry *et al.*(15). Paper electrophoretic analyses were performed on a Spinco Durham type cell. A constant current of 5 milliamperes for 17 hours was used. The dyed strips were run through a Spinco Analytrol for evaluation.

Results and discussion. Newborn calves studied had multiple defects which included progressive muscular dystrophy. Internal hydrocephalus associated with hypoplasia, dysplasia, disseminated amyelinogenesis of the

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TABLE I. Serum Enzyme Levels in Calves with the Hydrocephalic Syndrome and in Control Calves.

Group	Serum enzymes					Serum protein, mg/ml
	GOT, units*/ml	GPT, units*/ml	Aldolase, units†/ml	LDH, units‡/ml	MDH, units‡/ml	
Hydrocephalic syndrome (encephalo-ophthalmomyopathy)	4,400	660	385	9.0	4.6	34.0
	2,920	500	362	4.5	2.2	41.3
	1,960	260	265	3.6	2.1	41.0
	3,000	400	350	6.0	3.0	48.5
Means	3,070	440	340	5.5	3.0	41.2
Control	100	3.0	6.2	.18	.11	94.8
	38	5.0	6.4	.17	.02	46.0
	56	4.5	5.9	.11	.06	92.5
	96	4.5	3.8	.14	.13	50.5
	62	2.5	7.0	.15	.08	62.0
	66	3.0	6.2	.13	.05	40.5
Means	70	3.8	5.9	.15	.09	64.4

* Sigma Frankel units.
† Sibley-Lehninger units.
‡ Micromoles of DPNH oxidized/min at 25°C.

cerebellum with deformity of the mesencephalon, and bilateral cone shape detachment of the retina and cataracts were also apparent (10). Twelve calves with these defects were examined in detail. Eight of these were dead when received at the laboratory. Serum was collected for analyses from the 4 live calves.

All 5 of the serum enzymatic activities assayed were elevated in the serum from these calves as compared with control calves. The results are presented in Table I. MDH, LDH, aldolase and GOT activity averaged more than 30 times the level of the normal serum while the GPT activity averaged over 100 times higher. These elevated enzyme levels were probably due to liberation of these enzymes from muscle tissue as a result of the acute progressive muscular dystrophy since all of the enzymes assayed are known to occur in much higher concentration normally in muscle tissue than in serum. The proteins normally detected by paper electrophoretic analysis were all present in the serum of the calves with the hydrocephalic syndrome. Further, the proportion of each component did not appear to differ significantly from that of normal calves.

One of the defects in the calves studied was internal hydrocephalus. Since the blood-cerebrospinal fluid barrier results in enzyme activities that are relatively independent for serum and cerebrospinal fluid, it would be interesting to determine if any elevation in the above enzymes occurs in the cerebrospinal fluid. The defects occurring in these calves appear to be hereditary(16); hence, it would be desirable to determine the enzyme activities in the serum of the dam during pregnancy.

Summary. The serum enzyme levels were studied in 4 calves which exhibited a hydrocephalic syndrome (encephalo-ophthalmomyopathy(10)). More than a 30-fold elevation of serum glutamic oxaloacetic and glutamic pyruvic transaminases, lactic and malic dehydrogenases, and aldolase was found compared with sera from normal calves of similar age. The elevated serum enzymes, not previously reported for calves with the hydrocephalic syndrome, were believed to be due to extensive and progressive muscular dystrophy observed in these calves.

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Blood Plasma Adenosine Deaminase in Rats with Fibrosarcoma. (27907)

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Adenosine is deaminated in the animal organism and converted to inosine which by phosphorolysis produces hypoxanthine. When xanthine oxidase is present hypoxanthine is oxydized to xanthine and uric acid. The deamination of adenosine is catalyzed by the enzyme adenosine deaminase as follows:

$$\text{Adenosine} + H_2O \rightarrow \text{Inosine} + NH_3.$$

Adenosine deaminase is largely distributed in animal tissues chiefly in the intestine, muscles, kidneys and liver. In blood almost all the enzymatic activity is confined to the erythrocytes, blood plasma being a poor source of this enzyme. In pathological cases high values have been reported. Straub *et al.* (1) and more recently Letnansky and Seelich(2) have shown in cancer patients an elevated adenosine deaminase of blood plasma correlated to the degree of the disease. However, the increase in enzyme activity seems not to be specific as pointed out by Schwartz and Bodansky(3). Such other pathological conditions as miliary tuberculosis and hepatitis also presented high values for plasma adenosine deaminase.

The present study deals with the adenosine deaminase of the plasma of rats bearing a tumor induced experimentally. As far as we know there is a lack of references dealing with plasma adenosine deaminase in experimental neoplasia.

Material and methods. The tumor was originally induced in a male albino Wistar rat by one of us (L.A.A.) by subcutaneous injection of 1,2,5,6-dibenzanthracene(4) and was maintained monthly with subcutaneous implantation of 2-3 cu mm of tissue by means of a trocar. The tumor has developed well in all the animals during 2 years of regular transplants. Histological examination indicated it to be a fibrosarcoma.

Plasma adenosine deaminase was determined in male rats averaging 169 g in weight with tumor weighing 38 g (22% of total body weight). Fig. 1 shows the size of the tumor.

The rats were used 30 days after transference of the tumor. Normal rats of the same age and sex and maintained at the same balanced diet were used as controls (average body weight 164 g).

Blood was withdrawn by heart puncture after slight ether anesthesia and then collected in heparinized tubes. The plasma was immediately separated by centrifugation, hemolyzed plasma being discarded.

The enzyme determinations were performed by the methods of Koehler and Benz(5) and Kalckar(6). In the first method the ammonia released was estimated by Nessleriza-

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