pared to egg slant controls and increased growth over liquid charcoal cultures. The large race strains—200; K9; and Komin, a new strain—grew equally well in the diphasic blood medium. 3. The liquid blood medium was particularly unsatisfactory for maintenance of strains of small race, as the organisms were not easily discerned in the highly cellular sediment. 4. No attempts were made to culture stools or to isolate new strains of small or large race in either blood medium. 5. A 48-hour subculturing scheme was satisfactory for diphasic blood medium, liquid charcoal medium and egg slants; 0.5 ml of sediment was transferred.

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Some Alterations in Serum Enzymes in Progressive Muscular Dystrophy.* (23006)

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Considerable attention has been given recently to the increase in activity of serum glutamic oxalacetic transaminase associated with tissue destruction. Examination of rat tissues by Cohen and Hekhuis(1) and Awapara and Sealer(2) showed this enzyme to occur in highest concentration in heart muscle. Skeletal muscle has about 3/4, and liver about 3/5 of heart muscle activity. Thus it is not unexpected that in diseases involving these tissues, such as myocardial infarction (3), acute liver injury(4) and in several muscle disorders(5) there is an elevation of serum transaminase activity. Schapira *et al.* (6) found that in progressive muscular dystrophy there is an increase in serum aldolase. There are several possible explanations for the increase in serum enzyme concentration; muscle destruction, the enzyme may have its origin in tissue other than muscle, and acceleration of protein turnover may result in an increase of enzyme release, producing a new balance between serum enzyme and the mechanism for enzyme disposal. By concurrent determination of serum aldolase and transaminase we have attempted to examine the first two of these possibilities. In addition alkaline and acid phosphatases were determined as representatives of a third group of enzymes.

Methods. Serum glutamic oxalacetic transaminase was assayed to modification of the method of Karmen(7) developed by Steinberg *et al.*(8). Enzyme activity was expressed as micromoles of oxalacetic acid formed/hour/ml of serum at 37°. Serum aldolase was determined by the Dounce *et al.* (9) modification of the method of Sibley and Lehninger(10). Following the suggestion of Beck(11) we extended chromogen reaction time from 30 to 60 minutes. The substrate employed was the magnesium salt of fructose

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TABLE I. Comparison of Mean Values of Enzyme Activity in Normal Serum and Serum of Muscular Dystrophy Patients.

Enzyme	Children: Dystrophy 8, age 7-13	Children: Control 10, age 2-14	p	Adults: Dystrophy 9, age 15-54	Adults: Control 6, age 22-40	p
Alkaline phos., mM/l/hr	3.5 ± .80	3.3 ± 1.62	*	2.2 ± 1.46	2.1 ± .59	
Acid phos., mM/l/hr	.79 ± .18	.64 ± .30		.64 ± .20	.44 ± .06	.05
Aldolase, μg p/ml/min.	1.82 ± .43	.28 ± .10	.001	.55 ± .50	.19 ± .05	.001
Transaminase, μM/ml/hr	2.59 ± 1.80	.85 ± .27	.01	.86 ± .42	.65 ± .12	.05
$\frac{\text{Aldolase} \times 10}{\text{Transaminase}}$	5.6 ± 2.36	3.4 ± .79	.01	6.3 ± 3.49	2.8 ± .30	.05

* Where no value for p for difference is given, there was no significant difference.

diphosphate (Mann). It was used without further purification. At normal serum aldolase levels 1 ml of serum need be used, however with higher activity 0.5 ml is sufficient. Activity was expressed as μg of triose phosphate phosphorus formed/minute/ml of serum at 37°. Alkaline phosphatase was determined by the method of Bessey *et al.*(12), using 0.1 ml of serum. The acid phosphatase method used was that of Andersch and Szcypinski(13). Phosphatase units are expressed as millimoles of p-nitrophenol/liter of serum/hour.

Results. The cases were divided into 2 groups, adults and children. Average values, standard deviations and significance of differences between results in the 2 groups and the controls are given in Table I. Considering first the serum alkaline and acid phosphatase determinations, (Table I), there appears to be little or no alteration produced by muscular dystrophy. Our control sera were from adult laboratory personnel, and from hospitalized children with no neuromuscular disease and with aldolase and alkaline phosphatase activities within normal range(6,12). No information was available on normal range of acid phosphatase in children by the method we employed.

Our results for serum aldolase activity agree with the much larger series of Schapira *et al.*(6). In 61 cases of childhood muscular dystrophy they found a serum aldolase range of 0.3 to 13.0 with a mean of 3.3. We found a range of 0.37 to 4.08 with a mean of 1.82. Their values on normal children, mean 0.4 and range 0.2 to 0.6, are also in agreement with ours. On adult cases of muscular dystrophy we also found elevation in serum aldolase as reported by Schapira *et al.* Childhood muscular dystrophy is accompanied by higher serum aldolase activity than in the adult. This is in accord with previous observations(6,14). Siekert and Fleisher(5) determined serum transaminase on 3 cases of muscular dystrophy, presumably adults. They found an elevation of activity in 2 of them. Their controls consisted of 25 men and 25 women. The mean for females was 0.85 ± 0.177 and for males 1.16 ± 0.320, for the combined males and females 1.01 ± 0.298. They considered abnormal those activities that exceeded the mean plus 3 standard deviations. No adults with muscular dystrophy in our series can be considered abnormal by their criteria. Our own smaller control series had a lower transaminase activity range than that of Siekert and Fleisher. However there was no significant difference, $p = 0.05$, between means of control series and adult dystrophy cases. The mean activity for dystrophic children was 2.59 which is well above the normal range of either our own controls or the adult series of Siekert and Fleisher. There is a significant difference, $p = 0.001$, between the means for dystrophic and normal children.

The level of serum transaminase appears to be proportional to amount of heart muscle damage in myocardial infarction(15) and liver injury(16). It appears probable that elevated serum transaminase in muscular dystrophy similarly comes from skeletal muscle damage. Examination of data in Table I indicates there is a correlation between aldolase and transaminase activity, as aldolase

TABLE II. Aldolase and Transaminase Activities of Rat Tissues.

	Aldolase	Ref.	Transaminase	Ref.	Aldolase × 10 / Transaminase
Skeletal muscle	3440 mg/kg/min.	10	289 μM/g/hr	2	119.2
Heart muscle	620	10	334	2	18.5
Liver	560	10	250	2	22.4
Serum	2.8 mg/l/min.	18	11.1 μM/ml/hr	16	2.5

activity increases so does transaminase. This suggests that increased aldolase in muscular dystrophy is also derived from muscle.

It appeared of interest to calculate a factor that expressed the relative activities of serum aldolase and transaminase. This was done by multiplying the units of aldolase activity by 10 and dividing the product by transaminase activity. Our normal adult series had an A/T ratio ranging from 2.5 to 3.3, mean 2.8, Table I. The control children had an A/T ratio of 2.3, range 1.9 to 4.7. Relative to these controls, 13 of 17 muscular dystrophy cases showed elevations of the A/T ratio. The elevation was significant, $p = 0.001$, in the case of children but not in adults. In contrast to this are the results from the study of myocardial infarction by Siegel and Bing (17). They determined aldolase and transaminase in 5 cases of myocardial infarction and in 9 normals. We have recalculated their results to units we employed and the mean A/T ratio of their normals was 3.6, which is comparable to our A/T ratio of 2.8. From their data one finds that in myocardial infarction the A/T ratio falls below normal, in one case reaching 0.7. This suggests that in myocardial infarction more transaminase than aldolase is being released into the blood.

Concentrations of aldolase and transaminase in human tissues are not known. From several sources was assembled a Table of the aldolase and transaminase activities of rat tissues, recalculated to the same units used here (Table II). From these data it would appear that simply rupturing skeletal muscle cells would increase the A/T ratio of serum because of higher A/T ration of muscle, if it is assumed that both are cleared at comparable rates. Another source appears unlikely, especially since phosphatases, that do not occur in high concentrations in muscle, are not elevated, as might happen, for example, if liver, in which there is a high concentration of phosphatases, was the source for the extra aldolase.

An alternative explanation would be that aldolase and transaminase are removed from plasma at different rates. Sibley and Lehninger(18) confirming the observations of Warburg and Christian(19) found aldolase to be removed rapidly from plasma of rats. They injected crystalline aldolase and found serum aldolase elevated about 7 fold within 15 minutes, while 12 hours later the activity had fallen to only 1½ times normal value. Comparable experiments with transaminase have not been done. It appears, however, that transaminase is disposed of readily, since unless blood is sampled within 3 days after myocardial infarction, elevation in transaminase activity may be missed(20). The low A/T ratio in myocardial infarction may reflect the fact that the A/T ratio in heart muscle is only 1/6 of that found in skeletal muscle. Further data are needed from experiments in which human tissues and serum are analyzed by the same methods.

Summary. Acid and alkaline phosphatases, transaminase, and aldolase were determined in sera of 8 children and 9 adults afflicted with progressive muscular dystrophy. There was no alteration in phosphatase activity. Aldolase was increased in both children and adults while transaminase was distinctly elevated only in children. The ratio of aldolase to transaminase activity in serum increased in muscular dystrophy. The results indicate that increased activities of both aldolase and transaminase in progressive muscular dystrophy are derived from diseased muscle.

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Pressor and Myotrophic Activities of Incubated Plasma.*† (23007)

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Plasma from children in the hypertensive phase of acute glomerulonephritis was reported to have pressor activity which diminished or disappeared during recovery(1). These samples had been at room temperature for sometime; activity was increased by incubation at 37°C for an additional 12 hours, a procedure which elicited similar, but less pressor activity in samples from normal children. The possibility was raised that the pressor agent might be angiotonin, liberated because the plasma of hypertensive subjects contained increased amounts of renin.

Accordingly, a survey was done in which we tested in Dibenamine-treated, anesthetized rats, or pithed rats, the pressor activity of plasma from 28 patients with established hypertension, 14 patients in malignant phase of essential hypertension, 3 with hypertension proven to be of renal origin, 7 with hypertension and chronic glomerulonephritis and 6 normotensive patients with renal disease of systemic lupus erythematosus as well as 11 normal subjects. For these tests, blood was taken by Dr. Carlos Nijensohn under sterile conditions into heparin-containing tube, immediately centrifuged, plasma separated and divided in 2 fractions. One fraction was incubated for 24 hours at 37°C and the other frozen. Both were assayed at the same time, using angiotonin as the reference standard for pressor activity. Freshly frozen plasma in doses to 0.2 ml gave irregular and small pressor responses similar to those elicited by the same volume of saline. The incubated samples were all pressor, approximately in the same degree and without reference to presence of hypertension. In brief, the survey indicated that pressor activity in question, while apparently not of immediate significance in problem of hypertension, represented at least an interesting property of shed, pre-

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