of MAO inhibition on the metabolism of glucose by the human gland.

The finding that MAO activity of intestinal mucosa exceeds greatly that of any other tissue was not anticipated. In subsequent studies of the rat and the guinea pig, however, we found that a similar relationship exists. It is unfortunate that postmortem specimens of intestinal mucosa are unsuitable for study since this makes comparisons with the activities of other tissues within single individuals impossible.

The jejunal biopsy technic of Crosby makes it possible to obtain tissue specimens for assay of MAO activity frequently with minimal discomfort and essentially no risk to the subject. The use of a direct assay technic obviates sources of error inherent in indirect approaches such as measurement of urinary amines. The range of values for MAO activity in jejunal mucosa is sufficiently small so that it is not difficult to identify variations from normal when they occur in disease states or under the influence of various drugs. The very small range of variation in a single subject on different days makes studies involving the use of an individual as his own control most useful for evaluating the effect of a drug on MAO activity. We have had occasion to perform biopsies in hypertensive subjects as many as 10 times with all assays of MAO activity falling within a range of 12%. Preliminary observations on lowering of enzyme activity by MAO inhibiting drugs or in hyperthyroid states have shown this to be a very reliable and satisfactory technic for direct detection of spontaneous and induced variations of MAO activity.

Summary. The distribution of monoamine oxidase activity in human tissues was surveyed. The significance of this distribution and the differences between humans and various animal species were discussed. Direct assay of MAO activity in specimens of jejunal mucosa obtained from human subjects by peroral biopsy was shown to be feasible. In a series of normal subjects studied in this fashion the range of variation is sufficiently narrow to make this a useful procedure for detection of spontaneous and induced variations of MAO activity.

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Serum Enzyme Studies in Muscular Dystrophy. III. Serum Malic Dehydrogenase, 5-Nucleotidase and Adenosinetriphosphatase.* (27161)

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The assay of serum enzymes has become an established diagnostic procedure in clinical medicine. Commonly assayed enzymes, such as glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH), and aldolase, are widely distributed in mammalian tissues and are found in variable and, usually, in high concentrations in the liver and in cardiac and skeletal muscle. In normal subjects these enzymes are almost exclusively confined within the body cells where

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TABLE I. Values (in Units) of Serum Enzyme Activity.

Subject	5-nucleotide	ATP-ase pH 9.0	ATP-ase pH 4.5	MDH	GOT	GPT	LDH	Aldolase
Normal	.35 ±.29 (36)*	1.79 ±.75 (36)	1.80 ±.92 (28)	83.8 ± 29.0 (81)	16.6 ± 5.5 (111)	10.33 ± 4.6 (111)	230 ± 66.8 (80)	5.87 ± 1.97 (74)
Muscular dystrophy (acute)	.143 ±.13 (12)	1.61 ±.48 (17)	1.66 ±.53 (16)	300 ±159.3 (20)	89.9 ± 72.0 (43)	76.0 ± 54.7 (43)	1,220 ± 593 (38)	58.97 ±39.6 (34)
" (chronic)	.27 ±.16 (10)	1.11 ±.91 (11)	1.10 ±.57 (10)	109.0 ± 35.3 (16)	22.2 ± 13.1 (30)	15.8 ± 12.0 (30)	358.7 ± 125.7 (27)	9.72 ± 6.4 (26)

* No. in parentheses signifies No. of individual sera tested.

they carry out their physiologic functions and only a small amount is present in the circulation. It is believed that the concentration of serum enzyme in the normal individual reflects either a physiological breakdown of cells or an unavoidable escape through a partially permeable cell membrane. Elevated serum enzyme levels have been found in a variety of pathological conditions in which there is increased destruction of tissue. Striking increases in serum GOT, GPT, LDH and aldolase may be due to the rapid breakdown of a relatively large amount of cardiac, hepatic or skeletal tissue, each of which is rich in most of these enzymes. Previous reports (1,2,3,4,5,6,7) of increases in serum enzyme levels in muscular dystrophy suggested the probability that other enzymes may be released in abnormal quantities from dystrophic muscle. The possibility thus exists that a serum enzyme pattern which is characteristic of muscular dystrophy may be uncovered.

The present report is concerned with the study of the serum levels of as yet untested enzymes in dystrophy. These are malic dehydrogenase (MDH), 5-nucleotidase, and adenosinetriphosphatase (ATP-ase). As a reference point, 2 transaminases, lactic dehydrogenase and aldolase, were also estimated in all patients in these series.

Materials and methods. The 73 patients, of both sexes, drawn from the U.C.L.A. Muscular Dystrophy Clinic, ranged from 2 to 73 years in age. Forty-three were of the acute (childhood or pseudohypertrophic) type and 30 were the chronic (adult slowly progressive or myotonic) varieties. None of the patients had liver, cardiac, or renal disorders. Control sera for comparison were obtained from normal student volunteers, or were drawn from subjects who attended the Pediatric Clinic and who were without evidence of hepatic, cardiac, or muscle disease. The 79 males and 32 females in this group ranged in age from 2 to 33 years.

Blood was drawn by venipuncture and allowed to clot at room temperature for 1 to 2 hours. Great care was taken to avoid hemolysis which can liberate enzymes from erythrocytes and falsely elevate the levels of many serum enzymes. The clotted blood was

centrifuged at 3000 rpm for 10 minutes. The serum was separated and again centrifuged for an additional 10 minutes to remove any remaining cells. Most determinations were done on the same day the serum was obtained. A few sera were frozen in closed tubes at −20°C and determinations were made within 5 days and on only once-thawed serum.

5-Nucleotidase was determined according to the method of Dixon and Purdom(8) and serum ATP-ase activity was measured, both at pH 4.5 and 9.0, by the method of Meister (9). The results for 5-nucleotidase and ATP-ase were expressed as Bodansky units. GOT and GPT levels were determined by the method of Reitman and Frankel(10). The results were expressed as Karman transaminase units per 1 ml serum at 37°C. Aldolase was measured by the method of Bruns(11). Aldolase activity was expressed as the amount of fructose-1:6-diphosphate split into triosephosphate by 1 ml of serum in 1 hour at 37°C. LDH activity was determined by the method of Cabaud and Wroblewski(12). Each unit was equivalent to that amount of enzyme that would cause a decrease in $O.D._{340}$ of 0.001/minute in a reaction mixture of 3 ml volume per Wroblewski and La Due (13). The method of Siegel and Bing(14) was used for measurement of MDH. One unit of MDH was equal to an optical density change of 0.001/minute at 340 mμ.

Results. Table I summarizes the results of the present study.

Discussion. Neither 5-nucleotidase nor ATP-ase has been found to be altered significantly in the serum of patients with acute or chronic muscular dystrophy. Neither enzyme is a normal constituent of the muscle fibre. However, by histochemical methods 5-nucleotidase has been reported to be increased in quantity in the interstitial connective tissue in dystrophic muscle(15).

In the more rapidly progressive forms of dystrophy which usually occur in male children, high serum levels of GOT, GPT, LDH and aldolase have been noted by other workers. We have confirmed these findings in our patients. In addition, in this study malic dehydrogenase was also found to be consistently elevated in this form of the disease, although not at the same degree as were the other enzymes. A similar observation was reported by Dreyfus and his colleagues(16).

With the possible exception of aldolase, none of the tested enzymes was elevated in the serum in the chronic forms of muscular dystrophy or in myotonic dystrophy.

Previous studies(5,7,17) have shown that in acute or rapidly progressive dystrophy, these various enzymes may remain at the high levels in the serum for several years. As the disease advances and weakness becomes severe, serum enzyme levels decline and eventually reach the normal range. This is apparently due to a cumulative reduction in the mass of the functional muscle tissue.

The fundamental biochemical defect in the various forms of dystrophy has not yet been discovered. The continuous loss of serum enzymes from muscle indicates that an increase in permeability of the muscle fibre membrane exists. It is probable, although not proven, that this defect is secondary to another lesion within the muscle fibre itself. Since malic dehydrogenase normally resides in the mitochondria (sarcosomes) an increased rate of escape of this enzyme may signify that there exists either a permeability defect in the mitochondrial membrane or an enhanced breakdown of these structures in muscular dystrophy.

Summary. 5-Nucleotidase and ATP-ase were not found to be elevated in subjects with either acute or chronic muscular dystrophy. Patients with severe generalized rapidly progressive (acute) dystrophy had high serum levels of malic dehydrogenase as well as GOT, GPT, LDH, and aldolase. The patients with mild restricted slowly progressive (chronic) dystrophy had normal serum levels of most of these enzymes except that aldolase was slightly elevated in a few cases. It is suggested that an increase in muscle membrane permeability, probably a secondary lesion, allows a continuous leakage of these enzymes. An additional defect may exist in the mitochondrial membrane to permit the escape of malic dehydrogenase.

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Photodynamic Inactivation of the Vacuolating Virus, SV_{40}. (27162)

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The vacuolating virus, SV_{40}, isolated by Sweet and Hilleman(1), is a frequent contaminant of rhesus and cynomolgus monkey kidney cell cultures, in which it grows without any obvious cytopathic effect. The agent may be recovered by subculture on kidney cells from the green monkey, *Cercopithecus aethiops*, and in this system causes the cytoplasmic vacuolation from which its name is derived.

The prevalence of SV_{40} constitutes a serious problem in manufacture of live attenuated poliovirus vaccine, since existing federal regulations(2) will permit no detectable foreign virus in the product. Attention has been focused upon methods of differential inactivation which might be employed to kill SV_{40} (and perhaps other simian viruses as well) without destroying the infectivity of attenuated poliovirus.

In studying the inactivation of viruses by visible light in presence of photodynamic dyes(3,4) we were impressed by the remarkable selectivity of this process, and suggested (5) that it might be useful for differential inactivation of B-virus in suspensions of live poliovirus. The experiments described here were undertaken to determine whether or not SV_{40} is also amenable to differential inactivation by this method.

Methods. SV_{40}, strain 776, was obtained from Dr. Maurice Hilleman, who also kindly provided a supply of rabbit antiserum. Some of the experiments were performed with strain 777, isolated in this laboratory(6) from a sample of Salk poliomyelitis vaccine. The identity and purity of this strain were established by neutralization tests using the antiserum obtained from Hilleman.

The virus was propagated in monolayer cultures of trypsinized cercopithecus kidney cells in medium 199(7) with no serum added. The culture fluids were harvested after incubation for 5-6 days at 36°C and were centrifuged at 600 g to remove cell debris.

In certain of the experiments endogenous wild virus was collected by pooling the fluids from cultures of rhesus kidney cells containing tissue from 8 to 12 monkeys. The infectious agent in these fluids was identified as SV_{40} on the basis of the specific cytopathic effect on cercopithecus cells. For one experiment, SV_{40} was added so that a final concentration of 10^3 $TCID_{50}$/0.2 ml was present in a suspension containing $10^{7.3}$ PFU/0.2 ml of live poliovirus, type 1(LSC). The poliovirus