acid) for maintaining the integrity of the skin and a low permeability to water.

Summary. The fatty acid composition of total body fat of the gerbil was determined by gas liquid chromatography and compared to that of the rat. Oleic was the major fatty acid while in the rat linoleic and oleic were the predominant acids. Linoleic-1-C^{14} acid was administered orally as the methyl ester and C^{14} arachidonic acid isolated from total body fat. Stepwise degradation of the product revealed the synthesis of arachidonic acid, as in the rat, by the condensation of linoleate with an acetate moiety derived from linoleic acid.

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Glutamic Oxalacetic Transaminase Isoenzymes in Human Myopathies.* (29358)

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Glutamic oxalacetic transaminase (GOT) is widely distributed in mammalian tissues and is usually found in high concentrations in cardiac and skeletal muscles and in the liver. GOT has recently been shown to exist in two molecular forms, called isoenzymes, which can be separated by electrophoresis of sera and tissue extracts on suitable media(1, 2) or by column chromatography(3). An improved staining procedure for detection of GOT activity on starch gel has been developed(4,5). The sub-cellular distribution and the properties of these 2 isoenzymes have been studied in rat liver tissue(2). It was shown that the anodally migrating band (*i.e.* GOT I) was derived from the soluble supernatant fraction and the cathodal band (*i.e.* GOT II) from the mitochondrial fraction (2). It is known that the serum levels of GOT are often very high in certain human myopathies, especially in the childhood (Duchenne) type of muscular dystrophy(6,7,8), but it is not known whether this elevation is due to a composite of both molecular forms of GOT. A simple technic for semiquantitative determination of GOT isoenzymes, well-suited for routine clinical analysis, has been developed by combining agar gel electrophoresis, as described previously(9), and the staining procedure developed by Decker and Rau(4). The present report concerns a detailed study of GOT isoenzymes of sera and different tissues of normal humans and subjects with various myopathies.

Methods and materials. Biopsy specimens of anterior thigh muscle were obtained from 7 children with Duchenne dystrophy and 4 adults, 2 with periodic paralysis, one with dermatomyositis and one normal subject. Human skeletal muscle samples from various sites and from other organs were obtained at autopsy within 10 hours after death from 3 persons with diseases other than those of neuromuscular origin. The preparation of

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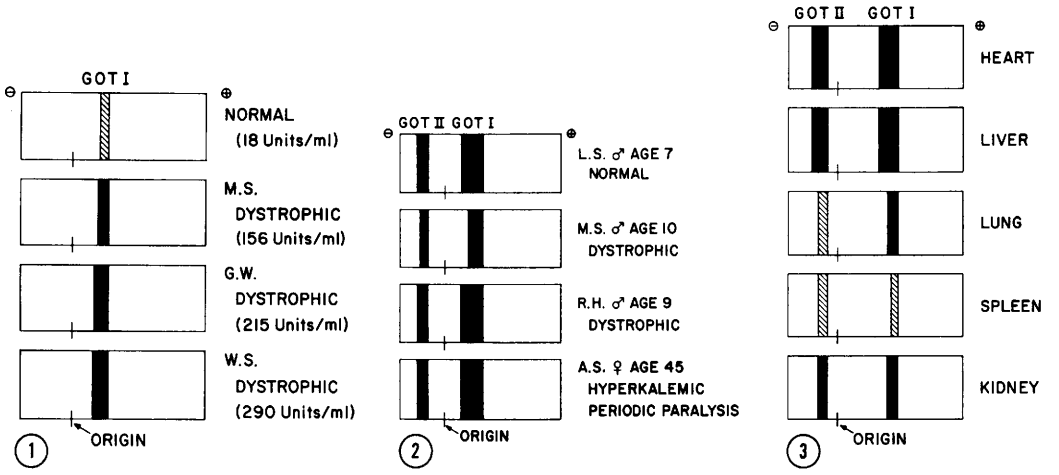

FIG. 1. Serum GOT isoenzymes patterns of normal and dystrophic subjects.
FIG. 2. Quadriceps muscle GOT isoenzyme patterns obtained from patients with several myopathies.
FIG. 3. Diagrammatic representation of GOT isoenzymes of certain human organs.

tissue extracts(9) and sera(7) was as described previously.

Twenty sera were studied, 17 from children with muscular dystrophy, one child and one adult with dermatomyositis and one adult with periodic paralysis. GOT levels of sera were determined by the method of Reitman and Frankel(10). The results were expressed as Karman transaminase units per ml of serum at 37°C.

Separation of GOT isoenzymes: Agar gel electrophoresis on ordinary microscope slides in barbital buffer, pH 8.6 of ionic strength 0.05, was performed as described previously (9). Twenty μl of serum or tissue extract was employed for isoenzyme analysis. Good separation of GOT isoenzymes could be obtained by carrying out the electrophoresis at a potential gradient of 6.5 volts/cm for 90 minutes at room temperature.

Localization of GOT isoenzymes: After electrophoresis the agar gels were stained for GOT activity according to the method of Decker and Rau(4). Following careful washing with 5% acetic acid and drying at 37°C the slides could be stored indefinitely. Due to orange background staining of the agar, the red-brown bands of GOT activity, although clearly visible and well delineated, did not photograph well so that diagrammatic representation of the patterns is used in the text.

Results and discussion. GOT isoenzyme patterns of representative sera obtained from normals and patients with childhood muscular dystrophy are shown in Fig. 1. The normal serum contains only a small amount of the fast moving band of GOT (*i.e.* GOT I). Sera obtained from patients with muscular dystrophy usually had a high GOT content. On agar gel electrophoresis this increase was shown by an intensification of staining (Fig. 1). This increase was due *only* to a rise in the GOT I fraction. In studies on 20 individuals with various myopathies we have not encountered any serum in which the cathodal GOT band (*i.e.* GOT II) appears, even when the serum GOT level has gone up to 290 units/ml (Fig. 1). Heterogeneity of serum GOT has, however, been reported by Rosa *et al*(11) but we have not been able to confirm this by our methods. According to these authors the general patterns were not different in normals or in dystrophic patients.

In Fig. 2 is shown the similarity of the patterns of GOT isoenzymes from anterior thigh muscles of a normal subject, 2 patients with childhood muscular dystrophy and one with familial periodic paralysis (hyperkalemic). In all, isoenzyme patterns were analyzed from thigh muscles of 11 patients, 7 of which had muscular dystrophy, one dermatomyositis, 2 periodic paralysis and one normal. The results in all were essentially simi-

lar. As would be expected, in advanced dystrophy the muscle enzyme content diminishes (*i.e.*, M.S. pattern in Fig. 2) but the general pattern or ratio of GOT I to GOT II does not recognizably change. In contrast to the multiplicity and diversity of the lactic dehydrogenase isoenzymes, even within a single tissue like striated skeletal muscle in human(9), GOT does not show characteristic differences in its pattern in various muscles of the body. Also GOT isoenzyme patterns show only minor variations from one tissue to another, and these are only in concentration of enzyme (Fig. 3). All of the organs studied contain both GOT I and GOT II. These isoenzymes are almost equal in content in heart, liver and kidney. Skeletal muscle contains somewhat less GOT II than GOT I activity (Fig. 2). In lung and spleen the total amount of enzyme is much less than that in heart, liver and skeletal muscle. GOT II has been shown to be derived from the mitochondrial fraction(2) so that the stronger concentration of this isoenzyme in heart and liver must be due to the greater number of mitochondria in these tissues than in the skeletal muscle and other tissues examined (12).

The observation that only GOT I is increased in the serum of dystrophic subjects implies that the elevated levels of GOT in these diseases are due almost solely to loss of the sarcoplasmic constituent of GOT, probably because of a defect in the cell membrane permeability, and that a major defect in the mitochondrial membrane, or destruction of the mitochondrial structure does not occur to any significant degree in muscular dystrophy or other myopathies which were studied. The physiological roles of each of these isoenzymes and physico-chemical differences of these two forms remain to be determined.

Summary. GOT isoenzymes of sera and muscle tissue from normal subjects and those with various myopathies have been analyzed by agar gel electrophoresis. Serum was found to contain only one fraction (GOT I) whereas muscle and other tissues contained variable amounts of 2 fractions (GOT I and II). The serum from persons with muscular dystrophy contained only an increased activity of GOT I fraction. Since this fraction is derived from the soluble sarcoplasm of muscle it implies that the mitochondrial fraction (GOT II) is retained and that mitochondria are not undergoing rapid breakdown in the myopathies. The proportional distribution of the 2 GOT isoenzymes in skeletal muscle tissue is unchanged in subjects with childhood muscular dystrophy and in other myopathies. GOT shows no difference in its pattern in skeletal muscle from different sites and only minor variations in certain other organs such as heart, liver, spleen, lung and kidney.

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