reaction between the various strains of *Trypanosoma cruzi* in that the serum from animals infected with one strain would, when tested, show staining of other strains of organisms. Also, cross infection could not be induced in guinea pigs as judged by parasitemia.

Using the indirect technique it was possible also to demonstrate that antibody was present. When test serum was placed on smears known to contain trypanosomes, staining with fluorescent anti-guinea pig gamma globulin yielded positive results. The serum from animals not infected, that is normal guinea pig serum, when so tested yielded negative results.

Discussion. This study indicates that antibody to *T. cruzi* is formed during infection in the guinea pig. It suggests that immunity is in part related to a circulating antibody. Cross-reaction between the 5 strains tested is to be expected as reported by others(3,4,5).

Convalescent serum conjugated to fluorescein may be useful in identification of organisms in smears and in tissue sections as both trypanosoma and leishmania forms can be stained. The fluorescent antibody technique may offer an additional tool in investigation of the pathogenesis of human Chagas' Disease. It may be that antigenic remnants that persist in the heart can be identified. This would furnish circumstantial evidence that although immune mechanisms are operative in the pathogenesis of the late stages of Chagas' Disease it need not be on an autoimmune basis.

Summary. Guinea pigs were infected with *Trypanosoma cruzi.* Five different strains were used and cross-immunity was evident between these strains. Serum from convalescent guinea pigs conjugated with fluorescein could be used as a stain for both the blood trypanosoma form and the tissue leishmanial form. Antibodies against the forms could also be identified using the indirect technique.

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Conversion of Linoleic-1-C^{14} Acid to Arachidonic Acid in the Gerbil. (29357)

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Relatively high levels of arachidonic acid have been found in rat plasma cholesterol esters by Howton and Hashimoto(2). Swell and associates(3) have confirmed this, and in a comparative study of the serum cholesterol ester fatty acid (CEFA) of 8 species of animals, suggested an inverse relationship between susceptibility to atherosclerosis and the arachidonic acid content of the cholesterol ester fatty acids. In the rat, which is relatively resistant to the production of atherosclerosis, the CEFA arachidonic acid content is very high (50%), whereas those species, *e.g.*, chicken, rabbit, pig, and guinea pig, whose CEFA contained relatively little arachidonic acid (1-2%) were readily susceptible to atherosclerosis.

Since the gerbil has been found to be resistant to atheroma formation(4), it might be expected on the basis of the above correla-

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tions that its CEFA arachidonate would be high. However, arachidonic acid is virtually absent and linoleic acid is the major fatty acid of gerbil CEFA(5). Thus it was of interest to us to ascertain whether the absence of arachidonic acid in the gerbil CEFA was due to inability to synthesize it or if this species represented an exception to the rule. Since the ability of the rat to convert exogenous linoleic acid to arachidonic acid has been demonstrated(6,7) we have used the same approach to determine whether the gerbil can perform this conversion.

Methods and materials. Five normal male gerbils weighing approximately 45 g, on Purina Lab. Chow diet and lettuce as a source of water, were each given an oral dose of 0.1 ml corn oil containing 0.1 mc and 1.7 mg of methyl linoleate-1-C^{14}.† Four hours later the animals were sacrificed and the organs and accessible adipose tissue removed and lyophilized. The dry tissue was extracted with absolute ethanol which was evaporated in a rotary evaporator. The lipid fraction was saponified in alcoholic KOH (7%) at room temperature under an atmosphere of nitrogen for 3 days. The nonsaponifiable fraction was removed by extraction with petroleum ether (30-60°C) and the fatty acids obtained by extracting with diethyl ether after acidification. For comparison, 2 male CFE strain rats‡ weighing approximately 120 g were sacrificed and organs and adipose tissue processed as described above. Fatty acid analysis was done on a Barber-Colman Gas Chromatograph, using a 6 ft × 4 mm column packed with 13% ethylene glycol succinate on 60-100 mesh siliconized chromosorb.

Since we were concerned with the isolation of gerbil arachidonic acid, the unsaturated fatty acids were concentrated by low temperature crystallization from acetone at −50°C(8). For greater stability, the unsaturated fatty acid fraction was hydrogenated in ethanol with 5% PdC and hydrogen at atmospheric pressure. The arachidic acid thus obtained was recovered by reversed phase chromatography on siliconized celite

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‡ Carworth Farms, New City, N. Y.

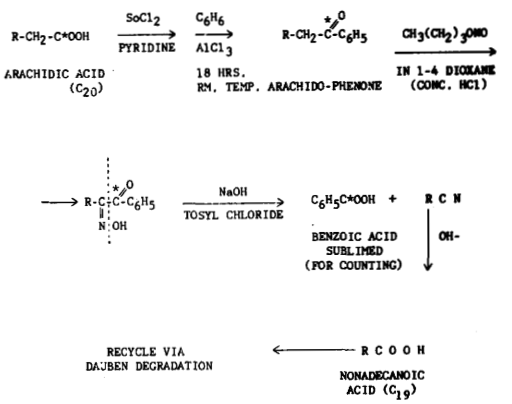

FIG. 1. Degradation of arachidic acid.

treated with mineral oil(7). The acid was isolated and recrystallized twice to yield 14 mg of C^{14}-labeled arachidic acid. This was diluted with 400 mg of carrier arachidic acid and subjected to stepwise degradation by a modification of the procedure of Dauben *et al* (7,9) shown in the flow diagram, Fig. 1. The arachidic acid was converted to the phenyl ketone by allowing its acid chloride to react with benzene and aluminum chloride under Friedel-Crafts conditions. The phenyl ketone was then nitrosated with butyl nitrite under acidic conditions and the resulting oxime rearranged in the presence of p-toluensulfonyl chloride and NaOH. The resulting C^{14} labeled benzoic acid was separated from the nitrile by means of silicic acid chromatography(7). The nitrile was hydrolyzed to the acid and the cycle repeated. The various carbon residues which were obtained as benzoic acid were counted in a Packard Liquid Scintillation Spectrometer.

Results and discussion. Table I shows the fatty acid distribution of gerbil and rat li-

TABLE I. Comparative Fatty Acid Distribution of Organ and Depot Lipids %.

Fatty acid	Chain length and No. of double bonds	Gerbil	Rat
Myristic	14:0	.7	2.7
Palmitic	16:0	24.5	17.8
Palmitoleic	16:1	2.8	8.3
Stearic	18:0	6.0	8.9
Oleic	18:1	47.9	27.0
Linoleic	18:2	13.5	31.8
Linolenic	18:3	1.2	—
Arachidonic	20:4	.8	3.5

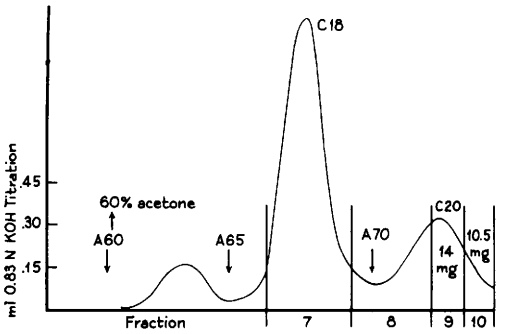

FIG. 2. Reversed phase chromatography of hydrogenated fatty acids on siliconized celite. Elution of components obtained by increasing concentration of acetone.

pids. These data were calculated from the gas-liquid chromatographic analysis of the saturated and unsaturated fatty acid fractions obtained by low temperature crystallization. The arachidonic acid content was relatively low in the gerbil, necessitating the concentration of the unsaturated fatty acids. The major fatty acids found were oleic, palmitic and linoleic, in that order. For comparison the fatty acid distribution of rat lipids is also included. The separation of the hydrogenated C_{20} and C_{18} fatty acids by reversed phase chromatography is shown in Fig. 2. Since it was most imperative that the labeled arachidic acid used for degradation studies be free of any other fatty acid, the arachidic acid peak was divided into 3 fractions, 8, 9, and 10, indicated in Fig. 2, and analyzed by gas chromatography. Analyses of these fractions revealed the presence of small amounts of C_{18} acid in fraction 8 and a small amount of C_{22} acid in fraction 10. Fraction 9, fortunately, was free of any contaminant and was used in the degradation studies.

Table II shows the specific activities of the various carbon residues. Carbons 1 and 3 are the most radioactive. From the activities of the various benzoic acid residues the percentage distribution of C^{14} in the different carbon atoms of the arachidic acid can be calculated. This is shown at the bottom of Table II. The high radioactivity found in carbons 1 and 3 with little activity in carbon 2 and the remaining C_{17} residue indicates that the gerbil can synthesize arachidonic acid from linoleate. These results are similar to those found in the rat(7), and present additional evidence that arachidonic acid is formed from intact linoleic acid to which is added a 2 carbon fragment derived, in part, from the first 2 carbons of linoleic acid.

Although oleic, linoleic and palmitic are the major fatty acids of gerbil lipids, oleic predominates. In the rat however, oleic and linoleic acids are present in about equal amounts. Evidently, the serum CEFA of these two species do not reflect the species tissue fatty acid patterns(2,3,5). These observations suggest a unique CEFA pattern for each species not only differing between species but also from its own total body fatty acid spectrum. The slight arachidonic acid content of the body fat and cholesterol esters simply indicates a minor role for this fatty acid in the lipid economy of the gerbil. Certainly the results reported here demonstrate the ability of the gerbil to synthesize arachidonic acid from linoleic acid. In fact, the high radioactivity in carbons 1 and 3 suggests a mechanism of biosynthesis very similar to that of the rat. Thus we must conclude that linoleic acid must be able to replace arachidonic acid in the gerbil in some of its functions and that the arachidonic acid synthesized in the body must be degraded or lost in some other way. It should be recalled, in this connection, that the gerbil, which normally requires very little water and retains it tenaciously, must depend on lipids containing polyunsaturated fatty acids of the linoleate family (presumably arachidonic

TABLE II. Specific Activity of Arachidic Acid and Its Degradation Products.

		cpm/μM
C_1-C_{20} Arachidic (C_{20})	537 cpm/ 5 mg	33.6
C_1 Benzoic	4943 cpm/39.5 "	15.3
C_2-C_{20} Nonadecanoic (C_{19})	467 cpm/ 7.1 "	19.6
C_2 Benzoic	204 cpm/16.7 "	1.5
C_3-C_{20} Stearic (C_{18}) (diluted x/5)	79 cpm/ 3.2 "	16.8
C_3 Benzoic (diluted 1/5)	65 cpm/ 2.2 "	18.0
C_4-C_{20} Heptadecanoic (C_{17}) (diluted 1/5)	10 cpm/ 7.8 "	1.7

$$\underset{4.7}{CH_3(CH_2)_{16}}\text{–}\underset{49.3}{C^*H_2}\text{–}\underset{4.1}{CH_2}\text{–}\underset{41.9\%\ C^{14}\text{-activity}}{C^*OOH}$$

3 2 1

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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