From the results tabulated in Table 1, there can be no doubt that *Leishmania enriettii* is capable of synthesizing α-linolenic acid.

The absence of radioactivity in fatty acids of chain length less than 18 carbon atoms strongly suggests that the unsaturated 18-carbon fatty acids and stearaldehyde were synthesized from stearic acid without degradation of the carbon chain and reutilization of 2-carbon units.

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Lactate Dehydrogenase in Testis: Dissociation and Recombination of Subunits

Abstract. *Electrophoretic resolution of lactate dehydrogenase in mature testes from a variety of animals revealed one or more unusual isozymes in addition to the usual five forms. Dissociation of the enzyme and recombination of the polypeptide subunits led to the formation of new isozymes and to a redistribution of activity among those normally present, indicating that lactate dehydrogenase synthesis in postpubertal testis is controlled by more than two genes.*

Five types of lactate dehydrogenase (LDH) have been identified in mammalian tissues by the method of starch-gel electrophoresis (*1*), LDH-1 being nearest the anode, and LDH-5 nearest the cathode. Evidence now available (*2*) suggests that each isozyme is composed of four polypeptide chains which are assembled from two different polypeptide units, A and B. Thus, isozymes 1–5 have the following polypeptide composition: A^0B^4, A^1B^3, A^2B^2, A^3B^1, and A^4B^0. If the synthesis of the A and B polypeptides is controlled by two different genes, then the isozymic repertory of any tissue or cell would depend on the relative activity of these two genes. Also it is evident that the maximal number of isozymes in any tissue would be limited to five. Our finding of six or more LDH isozymes in mature testes must, therefore, be related to the activity of additional genes.

Tissues were obtained immediately after death, except for human tissues which were obtained not later than 24 hours after death. Washed rabbit sperm were prepared from fresh ejaculates; bull sperm were concentrated from epididymes. Methods for preparation and electrophoresis of tissue homogenates and for localization of LDH isozymes in starch gel were those previously described (*3*). Dissociation of the LDH isozymes was accomplished by adding sodium chloride and phosphate, *p*H 7.0, to fresh homogenates in final concentrations of 0.5*M* and 0.1*M*, respectively (*4*). These mixtures were stored at a temperature of −20°C for 24 hours, thawed, and immediately subjected to electrophoresis. Measurement of LDH in these preparations with lactate as substrate revealed no loss of activity.

Analysis of LDH isozymes in testes from man, rabbit, dog, and mouse revealed one unique band ("band X") in addition to the usual ones (*5*). In the samples from man the electrophoretic position of "band X" was between LDH-3 and -4, in those from rabbit and dog, between LDH-4 and -5, and in mouse, behind LDH-5. Two bands of LDH "X" activity were seen in homogenates of guinea pig and rat testes, one between LDH-3 and -4, and the other between LDH-4 and -5. Preparations from bull testes exhibited three "band X" isozymes, one between LDH-3 and -4, and two between LDH-4 and -5. The positions of LDH isozymes in duck, chicken, hog, and cat testes corresponded to those of the isozymes in other tissues, and no electrophoretically distinct forms were evident.

To determine the relative amounts of the isozymes in mature human testes, each isozyme was eluted from starch and LDH activity was measured simultaneously with $2 \times 10^{-4}M$ pyruvate and reduced nicotinamide adenine dinucleotide ($NADH_2$) in one assay, and $2 \times 10^{-1}M$ lactate and nicotinamide adenine dinucleotide (NAD) in the other. "Band X" isozyme represented 11 percent of the total activity with pyruvate as substrate, and 12 percent with lactate as substrate. The individual contributions of LDH-1, -2, -3, -4, and -5 to total LDH activity were 12, 29, 36, 8, and 4 percent with pyruvate and 6, 27, 44, 8, and 3 percent with lactate.

Approximately 80 percent of the LDH activity in washed human, rabbit, and bull sperm resided in the "band X" area. Also in a series of 40 rabbits of different maturity "band X" activity did not appear until the onset of spermatogenesis at the 8th week of life. These observations suggest that "band X" material may play a significant role in sperm function.

In addition to the characteristic electrophoretic behavior of the "X" bands some, but not all, demonstrated unusual activity with α-hydroxy acids and with certain analogs of NAD. Allen (*6*) was the first to show that mouse testis and epididymis contained an isozyme which demonstrated greater activity with (DL) α-hydroxybutyrate and (DL) α-hydroxyvalerate than did the other isozymes. Since similar results were obtained with "band X" LDH from the mouse in this study, it appears that the unusual isozyme described by Allen is indeed "band X". The "X" bands of rat preparations demonstrated high activity with (DL) α-hydroxyvalerate. However, the "X" bands from man, bull, dog, guinea pig, and rabbit did not react with such α-hydroxy acids. As previously reported (*3, 7*), there is a high ratio of acetylpyridine adenine dinucleotide to NAD activity for "band X" from human testes and sperm. This was not observed in the other testes used in this study.

The unusual electrophoretic characteristics of "band X" from all sources studied and the unique catalytic properties of some raised the question whether these enzymes had been improperly classified as isozymes of LDH. In washed human, rabbit, and bull sperm approximately 80 percent of the conversion of lactate to pyruvate is accomplished by the "band X" enzyme, indicating that "band X" is the major form of LDH in sperm from these species. Further evidence for classifying "band X" as an LDH isozyme was obtained by dissociating and reassociating LDH in homogenates prepared from mature testes exhibiting "band X" activity. The results observed in human and rabbit testes are shown in Fig. 1. The untreated homogenate of human testis exhibits six major areas of LDH activity. After dissociation and reas-

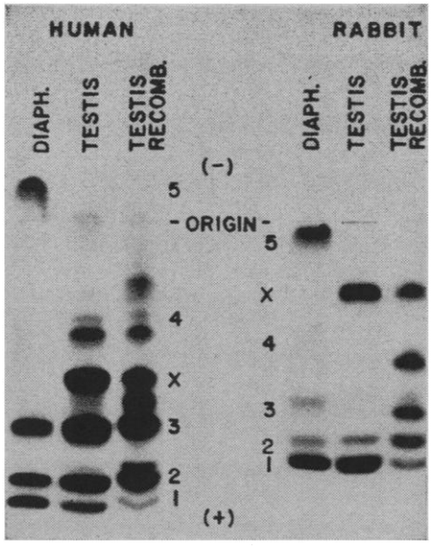

Fig. 1. Starch-gel electrophoretic patterns of LDH in homogenates of human and rabbit diaphragm and testis. The patterns of testicular homogenates which were treated with 0.5*M* sodium chloride and 0.1*M* phosphate and frozen for 24 hours prior to electrophoresis are designated "Testis Recomb." The total LDH activity and the amount of material subjected to electrophoresis were approximately the same for each tissue. Isozyme 4 sometimes appeared as a double or triple band. The LDH-5 band in human is very faint but the amounts actually present were large enough to combine with "band X."

sociation the activities of LDH-1, -2, -4, and -5 and "band X" decreased, and newly generated isozymes appeared between LDH-2 and -3, between LDH-3 and "band X", and between LDH-4 and -5. In the reassociation experiments with rabbit testes, the staining intensity of LDH-1, -3, and "band X" diminished, and new bands developed between LDH-2 and -3, and between LDH-3 and -4. The intensity of the band in the LDH-2 area increased. Studies of recombination of the "band X" material from guinea pig or bull testes revealed the development of three new isozymes whose electrophoretic positions were between those of LDH-1 and the slowest migrating component of the "band X" complex. After separation by means of diethylaminoethyl cellulose chromatography, the electrophoretic mobility of the recombinants in rabbit testis remained the same. No new isozymes appeared after dissociation and recombination of LDH in homogenates of rabbit muscle, heart, and diaphragm. In diaphragm, however, a redistribution of activity occurred among the isozymes normally present.

The changes occurring in testicular isozymic patterns under conditions which are known to dissociate LDH-1 and LDH-5 into monomers can best be explained by assuming that "band X" LDH is composed of neither A nor B subunits, but that it represents polymerization of new polypeptides which are formed by synthesis *de novo* or by chemical alteration of already existing subunits. In animals with a single "band X" the subunits are probably identical, so that its polypeptide composition may be designated as C^4. The isozymic complement of normal rabbit testis would be as follows: LDH-1 = B^4, LDH-2 = A^1B^3, LDH-3 = A^2B^2, and "band X" = C^4. The relative amount of each isozyme indicates that dissociation into subunits should yield large quantities of B and C, and a smaller amount of A. Random reassociation would favor the formation of BC recombinants, and isozymes of the following composition would be expected: B^4, B^3C^1, B^2C^2, B^1C^3, and C^4. If B and C are present in equal amounts, then the expected proportions of the isozymes would be 1:4:6:4:1. The distribution of activity in the recombinants of the isozymes from rabbit testis is compatible with this predicted ratio. The absence of new isozymes between "band X" and LDH-5 in rabbit testis is probably due to a deficiency in the number of A subunits with which C subunits can combine. The human testis, for example, contains a larger amount of LDH-4 and -5 so that reassociation between A and C would seem possible. As shown in Fig. 1, the presence of a new isozyme in human testis between LDH-4 and -5 suggests that one of these recombinants has indeed been formed.

The existence of multiple "X" bands in some animals suggests either that these isozymes are composites of more than one new polypeptide, or that the C polypeptide has combined with A or B subunits. Results of recombination studies on homogenates from guinea pig and bull testes support the first possibility. After treatment with sodium chloride the activity of the "X" bands decreased and three new isozymes appeared between LDH-1 and the slowest moving "band X".

An important question concerns the normal absence of isozymes composed of AC or BC polypeptides in untreated homogenates of rabbit and human testes. This observation implies either that the unlike polypeptides are physically incompatible or that the A and B subunits are unavailable for combination. The first explanation appears unlikely since recombinants can be formed in vitro. The possibility still exists, however, that the conditions for dissociation and reassociation alter the native "band X" material in such a way that combination with A and B subunits becomes possible. As previously noted, "band X" LDH is the major isozyme of thoroughly washed human and rabbit sperm. Hence the isozymic composition of the cell containing primarily "band X" greatly reduces the possibility of observing recombinants in vivo. Naturally occurring recombinants, however, might be seen when the genes controlling "band X" synthesis first become active. In the rabbit this would occur during the eighth week of life, and in the human at the time of puberty.

The group of LDH isozymes found in the tissues of those animals with a single "band X" can be explained on the basis of the activity of three genes, A, B, and C, each being responsible for the synthesis of a corresponding polypeptide. A unique feature of the C gene is that its activity is restricted to testis after the onset of sexual maturity. In animals with multiple "X" bands two new polypeptides appear to be present, thus suggesting that four genes participate in the synthesis of LDH in the testes of these animals (*8*).

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