Effects of Inanition, Protein Depletion and Repletion on Serum Lactic Acid Dehydrogenase Levels in Rats.* (24933)

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Since the initial report by Hill and Levi(1) on elevation of serum lactic acid dehydrogenase levels (SLD) in neoplastic disease, numerous clinical and experimental studies concerned with changes in SLD concentrations in a wide variety of pathologic states have been reported. In addition to cancer, increased enzyme levels have been observed in myocardial infarction, leukemia, anemia, diabetic acidosis, obstructive jaundice, infectious mononucleosis, hepatitis, muscular dystrophy, and other unrelated pathologic states(2,3). Inasmuch as cachexia, anorexia, and reduced food intake may be associated with above conditions, it was of interest to investigate the effects of level of dietary protein upon values of SLD. In the present study, the effects of inanition, protein depletion, and of repletion with diets containing 5 different levels of protein, on serum concentrations of SLD and total protein and on hematocrit values of blood have been investigated.

Materials and methods. Animals. Adult, male Sprague-Dawley rats were housed singly in suspended type wire cages with wire bottoms. They were maintained on Purina Laboratory Chow and tap water until initiation of the study. *Inanition.* Food was withheld from rats until they lost 25% of their original weight, the depletion requiring 8 days. Drinking water was available at all times. *Protein-free.*† Animals were fed the protein-free diet previously described(4) until they lost 25% of weight, the depletion requiring approximately 22 days. *Repletion.*† The 8, 17, 40 and 64% protein diets contained 10% vegetable oil, 4% salt mixture, (U.S.P. XIV), and the vitamin diet fortification mixture incorporated into the protein-free diet(4). The contents of casein and starch were adjusted to yield the desired protein level. For the 25% level of protein, ground Purina Laboratory Chow was employed(5). Rats were fed protein-free diet until they had lost 25% of weight. They were then repleted with diets containing the various levels of protein. When they had regained their original weight, the animals were bled. All diets were fed *ad lib. Chemical and hematologic analyses.* To avoid hemolysis, blood samples were obtained from the tail and centrifuged immediately for SLD determinations. Following this procedure, the animals were bled by cardiac puncture under light ether anesthesia. SLD activity was determined by the method of Hill and Levi(1), employing a Beckman Model DU spectrophotometer. Total serum protein and hematocrit determination were carried out by procedures previously reported(6). Analysis of data was made by standard statistical procedures employing the *t* test(7).

Results. The influence of dietary regimens on serum lactic acid dehydrogenase activity and other values is summarized in Table I. The results of hematocrit determinations indicated the development of hemoconcentration concomitant with depletion followed by a hemodilution during repletion. The effects were most pronounced in the starved group. The observations are in accord with the studies reviewed by Allison(8) which demonstrated that alterations in plasma volume may accompany depletion and repletion.

The general effects of fluctuations in plasma volume were to enhance the magnitude of the SLD changes and to minimize those of the total protein. Following a 25% weight loss produced by either starvation or protein depletion, significant elevations occurred in SLD concentrations. When the changes in optical density were corrected for increased hema-

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† Obtained from Nutritional Biochemicals Corp., Cleveland.

TABLE I. Effects of Inanition, Protein Depletion and Repletion on Serum Lactic Acid Dehydrogenase Levels.

Group	No. rats	Initial wt (g)	Depleted wt (g)	Repleted wt (g)	Hematocrit (%)	Total serum protein (g %)	SLD (ΔO.D. $\times 10^3$)
Normal	26	407 ± 4.4			45 ± .4	5.8 ± .06	17 ± 2.5
Inanition	22	404 ± 6.1	306 ± 4.6*		53 ± .6*	5.2 ± .08*	39 ± 3.2*
Protein-free diet	19	403 ± 6.6	304 ± 5.2*		47 ± .5*	5.0 ± .06*	28 ± 4.6*
8% protein	16	403 ± 5.8	306 ± 4.5*	400 ± 6.3	43 ± .5*	5.5 ± .07*	36 ± 6.8*
17% "	18	399 ± 5.8	304 ± 4.1*	401 ± 5.6	43 ± .8*	6.5 ± .09*	10 ± 2.4*
25% "	12	407 ± 3.3	308 ± 2.8*	408 ± 3.6	41 ± .6*	6.1 ± .06*	12 ± 2.1*
40% "	15	405 ± 3.6	303 ± 3.3*	400 ± 3.9	40 ± .4*	6.2 ± .08*	13 ± 1.2*
64% "	17	406 ± 2.6	309 ± 2.2*	406 ± 3.3	43 ± .5*	6.1 ± .07*	10 ± 1.8*

Findings include stand. error of mean.
Statistically significant differences from normal values are indicated: * P = <.01.

tocrit by the formula:

$$\frac{(100-H_2)\ (\text{observed}\ \Delta\ \text{O.D.})}{(100-H_1)}$$

in which H_1 = normal hematocrit and H_2 = experimental hematocrit(9), the differences were still highly significant. Upon repletion, SLD levels declined to less than normal in all but the 8% protein group. The increased SLD activity of this group was consistent with the decreased serum protein concentration but the cause was not apparent. SLD levels in some patients with cancer have also declined following administration of large amounts of protein(10).

The major factor contributing to the increased SLD levels was apparently degradation of somatic tissue, with release of the enzyme into the circulation. It is well established that skeletal muscle which quantitatively undergoes the greatest loss in weight during depletion(11) contains large amounts of lactic acid dehydrogenase(12). This interpretation is in accord with the clinical observations of White(3) who determined SLD and creatine excretion.

Subnormal concentrations of SLD following weight repletion on an adequate protein diet may be due to several factors: (a) in part to hemodilution (b) tissue protein may compete with SLD for common precursors (c) preferential synthesis of tissue protein during repletion (d) increased anabolism during repletion may increase tissues demand for SLD, resulting in release of reduced amounts to serum. The significant increases in total protein concentrations after repletion provide evidence that the mechanisms for protein synthesis were not injured during the depletion regimens. Increased concentrations of SLD in pathologic states, characterized by inflammation or abnormally rapid cellular growth, suggest that mechanisms other than tissue degradation may also play a role in increased SLD concentrations(2).

Summary. The effects of inanition, protein depletion and repletion upon serum lactic acid dehydrogenase (SLD), total serum protein and hematocrit values have been determined in adult, male Sprague-Dawley rats. Significant increases in concentration of SLD concomitant with decreases in serum protein levels followed inanition and protein depletion. Upon repletion, subnormal SLD values occurred in groups fed a diet containing 17% or more of protein. Serum protein values for the groups were significantly increased. The results are discussed with respect to possible causes for observed changes.

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Rapid Reversal of Ethionine Inhibition of Enzyme Induction in *Pseudomonas aeruginosa* by L- and D-methionine. (24934)

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A number of amino acid analogues are incorporated into bacterial protein when the cells are grown in their presence (*cf.* 1 for review of literature). Presumably this also occurs when enzymes are induced in resting cell suspensions although proof is lacking. Enzyme induction is inhibited by amino acid analogues in such cells and the appropriate amino acid when added with the analogue prevents the inhibition. It was of interest to determine how rapidly an amino acid can reverse the analogue inhibition once it is established. This would give an indication of the rate the analogue can be displaced from the site or sites to which it is attached.

Methods. A strain of *Pseudomonas aeruginosa* was grown for 24 hours at 35° in 100 ml Difco nutrient broth. The cells were centrifuged, washed in water and centrifuged again. They were then suspended in 12 ml of 0.05 M Na-K-phosphate pH 7.6, and 0.5 ml of this suspension was used in each Warburg vessel which had a final fluid volume of 2 ml. Oxidation of 0.5 mg Na benzoate was measured. This proceeds by simultaneous induction of several enzymes(2). Because of the number of enzymes involved and the complexities of oxidative reactions no conclusion can be drawn about the locus of action of the analogue. But oxygen uptake measurements can accurately detect changes in enzyme activity and the rate at which they occur.

Results. Fig. 1 shows results with DL-ethionine. It was added with the benzoate at zero time and after 90 minutes produced a constant 63% inhibition of oxygen uptake. Oxidation of benzoate alone began after a 30 minute latent period and reached a linear rate after 90 minutes. After 120 minutes L- or D-methionine was added. When added to the vessels containing only benzoate the 2 isomers had no effect on the rate. Ethionine added at this time was also without effect. When the 2 isomers were added to the vessels in which ethionine had been present from the beginning rapid reversal occurred. For the L-isomer this became evident in 15 minutes, almost complete during the next 15 minutes. For the D-isomer, reversal was not apparent in the first 15 minutes but occurred rapidly during the next 15 minute period. The initial difference between the 2 isomers was small but consistent. Exactly the same results were obtained with 1.0 μg p-fluorophenylalanine and 0.2 μg of L- or D-phenylalanine. Phenylpyruvic acid is also an antagonist for this analogue and it behaved initially like the D-isomer. Addition of amino acids other than the specific ones failed to reverse the inhibitions.

Three other facts may be briefly mentioned. If the methionines were added 60 minutes before ethionine and benzoate no reversal was evident presumably because both the L- and D-isomers were metabolized. On the other hand, if the amino acids were incubated 60 minutes with ethionine before the addition of benzoate reversal occurred presumably because ethionine had inhibited the metabolism of both isomers. Finally, if the nitrogen pool was increased by a prior incubation of the cells with 30 μg $(NH_4)_2SO_4$ and 4.3 μg succinic acid, ethionine inhibited to the same extent as in the untreated cells. Similar results were obtained with p-fluorophenylalanine.

Discussion. No conclusions can be drawn from these experiments about the site of action of ethionine but several possibilities may