The fact that enzyme treatment produced no increase in cell fragility may be interpreted in one of two ways: 1. There is no substrate for trypsin and papain in the concentrations used at the cell surface of strain L fibroblasts. 2. There is a protein substrate for these enzymes at the cell surface, which was hydrolyzed by the enzymes, but this protein does not play a significant role in imparting strength to the membrane (as measured by osmotic fragility).

Summary. 1. A method is described for measuring osmotic fragility of cells containing no visible pigment and available in limited quantities only. 2. By this method it was found that osmotic fragility of Earle's strain L fibroblasts is considerably less than that of mammalian erythrocytes, fibroblasts lysing at approximately 13% of isotonicity and erythrocytes at approximately 50%. 3. Microscopic observations are described suggesting that the cell membrane of Earle's strain L fibroblasts is an expansile structure. 4. Treatment of strain L fibroblasts with trypsin or papain produced no increase in their osmotic fragility.

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Effect of Vit. E-Deficiency and Dietary Glycine on Tissue Phosphatase Activity.* (23934)

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Carey *et al.*(1) reported that alkaline phosphatase activity of muscle homogenates from dystrophic rabbits was unchanged from that of control animals, whereas a 2-fold increase in muscle acid phosphatase activity from dystrophic rabbits was observed. White and Hess(2) found no alteration in serum phosphatase activity in children and adults afflicted with progressive muscular dystrophy. Tallan(3) showed an increase in level of most free amino acids in muscle extracts of dystrophic rabbits except for the concentration of glycine which was markedly reduced. This was later confirmed by Smith and Nelson(4) who in addition showed that glycine was consistently reduced in 4 other tissues of dystrophic rabbits. It seemed desirable to determine what effect, if any, the dystrophic condition as well as dietary glycine would have on enzymic activity of tissue other than muscle. Our studies describe the acid and alkaline phosphatase activity in liver, kidney, heart, spleen, brain, and muscle of normal and Vit. E-deficient rabbits, and the influence of dietary glycine on phosphatase activity of these tissues.

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Methods. Twelve male rabbits, divided into 4 equal groups, were fed the following diets *ad lib.*: A. Control diet 11 of Goettsch

TABLE I. Acid and Alkaline Phosphatase Activity of Tissues from Normal and Vit. E-Deficient Rabbits. Values expressed as μM P released/g dry tissue and probable error of the mean.

	Alkaline phosphatase (pH 9.0)				Acid phosphatase (pH 4.5)			
Tissue	Diet A Control	Diet B Deficient	Diet C Control + glycine	Diet D Deficient	Diet A Control	Diet B Deficient	Diet C Control + glycine	Diet D Deficient + glycine
Liver	102 ± 1.6	79 ± 4.2	104 ± 7.2	98 ± 19.5	122 ± 2.7	90 ± 2.6	123 ± 6.8	109 ± 4.1
Kidney	740 ±77.8	371 ±39.5	534 ± 3.1	691 ± 69.0	323 ±46.6	209 ±13.2	207 ± 7.2	234 ± 6.6
Spleen	675 ±41.1	464 ±78.2	579 ±98.4	682 ± 47.0	638 ±15.5	379 ±29.0	528 ±84.9	624 ±10.4
Brain	123 ± 4.0	85 ± 1.2	100 ± 3.5	111 ± 1.4	111 ± 5.1	84 ± 3.2	62 ± 7.2	96 ±14.8
Heart	146 ±35.5	53 ± 3.0	68 ± 4.0	65*	233 ±46.0	86 ± 3.8	106 ± 8.4	100 ± 2.0
Muscle	25 ± 5.8	23 ± 6.3	23 ± 3.8	25 ± 5.5	123 ± 8.8	84 ± 7.6	75 ± 1.8	83 ± 0.9

* Single determination.

and Pappenheimer(5); B. Dystrophy producing diet 11 which had been treated with 1% ethereal $FeCl_3$(5); C. Control diet 11 plus 1% glycine; D. Dystrophy-producing diet 11 plus 1% glycine. Controls also received oral supplements of 4 mg of alpha-tocopherol acetate/kilo body weight twice weekly. When symptoms(4) appeared, the animals were sacrificed. All deficient animals developed dystrophy at about the same time although those receiving glycine supplement exhibited fewer outward signs. One of the deficient animals died after 6 weeks and was excluded from the study. As soon as animals were sacrificed, samples of tissue were immediately homogenized with Teflon pestle in distilled water. The incubation system for phosphatase activity contained in final volume of 11 ml, 9 ml of buffer (veronal-acetate, pH 4.5, or veronal, pH 9.0), 1 ml of substrate (15×10^{-3} moles of $MgCl_2$), and 1 ml of homogenate. Incubations were carried out in small Erlenmeyer flasks with shaking in constant temperature bath at 37°C. After one hour, the reaction was terminated by addition of 5 ml of 20% trichloroacetic acid. Inorganic phosphorus was determined in a similar manner except that substrate was omitted. A 1 ml aliquot of each homogenate was removed and kept at 100°C for 24 hrs for determination of dry weight.

Results. Distribution of acid and alkaline phosphatase activity in various tissues of normal and dystrophic rabbits is shown in Table I. Values are expressed as μM of P released/g of dry tissue, and represent average of tissues from 3 rabbits, except for 2 animals on Diet D. There was a definite decrease in both acid and alkaline phosphatase of tissues of dystrophic animals on Diet B as compared to controls on Diet A. Kidney and heart tissue showed the largest decrease (50 and 64% respectively) at pH 9.0; whereas heart and spleen were most affected at pH 4.5 (63 and 40%). Only muscle homogenate at pH 9.0 showed no significant decrease. In contrast to these findings, tissue homogenates of animals on dystrophy-producing Diet D + 1% glycine showed no decrease in either acid or alkaline phosphatase activity as compared to their controls on Diet C.

Discussion. Most enzyme assays on Vit. E-deficient animals have naturally been carried out on muscle homogenates since this is the most obviously affected tissue. Jacobi *et al.* (6) found no differences in the activities of 6 enzyme systems between dystrophic and normal muscle. The same was true for uricase and adenosinetriphosphatase activities of liver homogenates from normal and dystrophic animals. Carpenter *et al.*(7) reported that dystrophic rabbit muscle has normal phosphoglucomutase activity and glycolysis.

Our results are in agreement with Carey *et al.*(1) with respect to alkaline phosphatase; however we found a slight decrease in acid phosphatase. Furthermore our results showed a significant decrease in both acid and alkaline phosphatase activity of 5 other tissues of dystrophic animals. Perhaps our most striking finding was that dietary glycine prevented decrease in tissue phosphatase of animals on Vit. E-deficient diet. Ray & Sen(8) reported that starvation decreased phosphatase activity of tissues, and that several dietary factors, including glycine, were effective in its regeneration. It should be emphasized, however, that our animals were not starved,

even the dystrophic animals continued to eat and show a weight gain until their sacrifice.

It is recognized that there is a disturbance of creatine metabolism in Vit. E-deficient animals. As early as 1932(9) attempts were made, without success, to treat progressive muscular dystrophy by supplementation with glycine on the assumption that there was a disturbance in formation of creatine. Dinning and Fitch(10) have recently reported that rate of creatine synthesis and turnover rate of skeletal muscle creatine is increased in dystrophic animals. Nevertheless that glycine decreases in most tissues of dystrophic animals and dietary glycine appears to prevent a decrease in tissue phosphatase, even though this enzyme has not been implicated with the dystrophic state, suggests that glycine is in some unknown way implicated in the dystrophic state.

Summary. 1. Acid and alkaline phosphatase activity of 6 tissues of normal and Vit. E-deficient rabbits was determined. 2. These activities were decreased in heart, spleen, liver, kidney and brain of dystrophic animals. 3. Acid phosphatase decreased slightly in muscle, but no change occurred in alkaline phosphatase activity. 4. 1% glycine in the dystrophic diet prevented reduction of tissue phosphatase.

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Enzyme and Nucleic Acid Content of Thrombocytes from Normal and Thrombocytopenic Calves.* (23935)

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Hypoplastic or aplastic anemia can be elicited in the bovine by feeding of trichloroethylene-extracted soybean oil meal (TCE-SOM)(1,2). Development and course of this anemia indicate that in this species the bone marrow is the primary site of action of the toxic agent. Among circulating derivatives of bone marrow, the composition of thrombocytes has apparently not previously been studied in any hypoplastic anemia.

Calves with induced hypoplastic anemia provided sufficient blood for preparation of thrombocyte concentrates in which the presence of nucleic acids and the activity of some hydrolytic enzymes could be investigated. Study of the latter was selected because sensitive analytical methods could be adapted for this purpose and because their activity in leukocytes is affected in other types of blood dyscrasias(3).

Methods. Young female Holstein calves used for other purposes(2) served as blood donors. Normal control calves were fed

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