ous neoplastic and non-neoplastic diseases may also prove fruitful.

Skeletal muscle and brain tissue of mouse were also pointed out as comparatively rich sources of LDH(6); however, there is no comparable study in human beings. If this finding could be applied to man, then gangrene of the lower extremity should give more striking results than myocardial infarction, and cerebro-vascular accidents should give data comparable to the latter. However, a normal level of serum LDH was noted. This might be explained as follows: The enzyme released from the necrotic tissues in the myocardium have ready access to the flowing blood stream in the cardiac chambers, thus resulting in an elevated serum LDH. Whereas in the leg and brain when occlusion of the major channel of blood occurs, the released enzyme does not have ready access to a blood supply, so the enzyme is destroyed or inactivated before reaching the circulatory system.

Our present studies did not confirm the elevation of serum LDH levels in diabetic acidosis as reported by Wróblewski and LaDue (2). Two of the 10 diabetic patients in the present investigation were in acidosis and the highest value obtained was 21.4. This might be due to the difference of degree in diabetic acidosis.

The mechanism by which serum LDH is increased in association with certain diseases is yet unknown. Experiments relating to the etiology may lead to a clearer understanding of this phenomenon. At present serum LDH determination may be used as a supplementary tool for diagnosing certain neoplastic diseases. It is a good index for myocardial infarction and diseases involving hepatic change.

Summary. The serum lactic dehydrogenase activity of normal human subjects and of patients with various diseases has been studied. Elevated levels were found in such neoplasms as stem cell and granulocytic leukemias, lymphomas, carcinoma of the pancreas and gallbladder, metastasis from carcinoma of the breast, and in metastatic disease of the liver. Increased enzyme activity was also noted in such non-neoplastic diseases as myocardial infarction, infectious mononucleosis, thrombocytopenia, acute infectious hepatitis and obstructive jaundice. Possible mechanisms of such elevation in serum LDH level have been discussed.

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Methemoglobinemia Induced by X-Irradiation.* (22354)

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As a first approximation it may be assumed that X-irradiation produces ion-pairs in aqueous solution as in air and for the same dose that the number of ion-pairs produced per unit weight is the same in solution as in air. A one-roentgen dose of X rays, by definition, produces 2.08×10^9 ion-pairs per 0.001293 g of air and may be considered to produce 1.6×10^{12} ion-pairs per ml of aqueous solution. Also, in aqueous solution containing oxygen there is reason to assume production of oxidant equivalent to 4 univalent atoms per ion-pair. Thus, one roentgen produces the equivalent of 6.4×10^{12} univalent atoms of oxi-

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dant per ml of solution. Since one microequivalent corresponds to approximately 6 x 10^{17} univalent atoms, a one-roentgen dose of X rays will produce the order of 10^{-5} microequivalents of oxidant per ml of solution. This estimate of the oxidizing ability of X rays is based on the simplest assumptions about mechanism and probably is somewhat low(1); however, the observed degree of oxidation of most biological materials is less than expected from the estimate(2). A recent report(3) indicates that 25,000 roentgens of X rays applied to lysed human erythrocytes diluted 1:40 with phosphate buffer converted about 60% of the hemoglobin to methemoglobin. For present purposes erythrocytes may be considered to contain 20 microequivalents of ferrous iron in the form of hemoglobin. The solution of hemoglobin irradiated contained, therefore, 0.5 microequivalents of hemoglobin iron per ml. The dose of irradiation used would provide 0.25 microequivalents of oxidant per ml and might, therefore, convert 50% of the hemoglobin to methemoglobin. The conversion reported, 60%, although large compared to results obtained with other biological material(2), is about the same as might be expected from the oxidizing potential of the radiation. The report(3) also indicates that irradiation of the rat *in vivo* with 3000 roentgens led to the conversion of about 2% of the hemoglobin to methemoglobin. This represents oxidation of 0.4 microequivalents of hemoglobin per ml of erythrocytes. A second dose of 3000 roentgens given immediately after the first led to the oxidation of additional hemoglobin. The amount oxidized by the second dose was about twice that obtained with the first dose. 3000 roentgens would provide 0.03 microequivalents of oxidant per ml of erythrocytes and might, therefore, convert 0.15% of the hemoglobin to methemoglobin. Thus, the amount of hemoglobin found oxidized was 13-26 times as much as expected.

It is possible that practically all of the oxidizing potential of the radiation arising throughout the animal appeared in the erythrocytes by way of the circulation. Assuming the animals had a total volume of 300 ml, a red-cell volume of 15 ml, and all of the oxidant arising from 3000 roentgens eventually appeared in the red cells, each ml of the cells would be subjected to 0.6 microequivalent of oxidant. This amount is of the same order as the amounts of hemoglobin found oxidized by 3000 roentgens. An alternative explanation is that the effect of the irradiation *in vivo* was multiplied in some fashion. The relatively small chemical potential of a dose of radiation that will profoundly affect an animal, *e.g.* 1000 roentgens, has given rise to speculation about the possibility of a multiplication factor, *e.g.* chain reactions. No biological system considered *in vitro* has shown the degree of multiplication implied by the experiments under consideration(2).

Experimental. The preceding considerations appear in order although in the present work using another strain of rat given up to 5000 roentgens of whole-body X-irradiation (250 KVP, 0.5-2.8 mm Cu HVL) essentially no methemoglobin was detected in the blood. Test for methemoglobin was made as follows: Immediately after irradiation the animal was anesthetized with ether. Blood was taken from the heart in heparin. An equal volume of cold water was added. The mixture was kept in the cold for about 15 minutes. An equal volume of cold saturated ammonium sulfate, adjusted to pH 6.6 with fixed alkali, was added. After standing 15-20 minutes in the cold, the mixture was filtered. The clear filtrate was assayed spectrophotometrically for methemoglobin(4). In addition, the filtrate was tested for change in optical density at 630 mμ following addition of cyanide(5) and was examined with a hand spectroscope for the characteristic absorption band of methemoglobin.

In both irradiated and control rats the level of methemoglobin did not exceed 0.2% and no difference between the groups was observed.

Summary. Methemoglobinemia was not observed in rats given up to 5000 roentgens of whole-body X-irradiation *in vivo*.

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Influence of Vit. E Deficiency in Rabbit on Urinary Excretion of Free Amino Acids.* (22355)

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It has been shown that urinary excretion of free amino acids is elevated in patients with progressive muscular dystrophy(1). Also it has been reported that vit. E-deficient rabbits excrete extra quantities of free amino acids in urine although no actual data have been presented(2,3). In view of these considerations, and since any information concerning metabolic disturbances in vit. E deficiency may aid in elucidation of the biochemical role of the vitamin, we have determined the influence of vit. E deficiency on urinary excretion of amino acids by rabbits.

Methods. White New Zealand rabbits, weighing initially about 500 g each, were given the same purified diet previously described(4) except that folic acid was omitted. Controls received this diet with oral supplements of 10 mg of alpha tocopherol acetate 2 times per week. There were 4 rabbits in each group. The animals were kept in individual metabolism cages and daily urine samples were collected throughout the experiment. Urine samples were analyzed for free amino acids by the copper method(5). The experiment was terminated when unsupplemented animals exhibited early stages of muscular dystrophy.

Results. Vit. E deficiency led to a considerable increase in excretion of free amino acids in urine. At the time of appearance of earliest physical signs of muscular dystrophy urinary excretion of free amino acids was tripled (Table I). Actually there was a significant increase in excretion of amino acids before any physical signs of dystrophy were apparent. This agrees with the observation of Ames (2).

TABLE I. Influence of Vit. E on Average Daily Urinary Excretion of Free Amino Acids by Rabbits.

Days on diet	mg amino N/kilo body wt	
	Without vit. E	With vit. E
1- 5	6.6	5.2
6-11	5.9	4.5
11-15	4.5	4.3
16-20	5.7	3.7
21-25	8.6	3.3
26-31	13.5	4.5

Increased excretion of free amino acids in vit. E deficiency may indicate an impaired incorporation of amino acids into protein, an increased breakdown of tissue proteins, or an impairment in normal metabolic disposal of amino acids. Experiments with C^{14} labeled glycine have indicated that vit. E deficiency does not significantly affect incorporation of that amino acid into protein or its oxidation to CO_2(6). This would suggest that elevated excretion of free amino acids in vit. E deficiency is due to accelerated breakdown of tissue protein, probably primarily skeletal muscle.

Summary. Vit. E deficiency in the rabbit results in increased excretion of free amino acids in urine. This increase in amino acid excretion is apparent before any physical signs of dystrophy are observed.

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