agent is inactivated only by a very large amount of ionizing radiation, which suggests a small target size and a molecular weight as low as 10^5 (*6*).

In apparent contrast with these data, filtration studies indicate that the size of the scrapie agent is 20 to 40 nm (*7*), thus well within the range of the particles described in this report and in experimental scrapie.

It has been proposed that an abnormality in the replication of cell membranes might be responsible for scrapie (*8*). Our finding of multiple cytoplasmic projections and vesicles budding inside neuronal vacuoles is compatible with this hypothesis. The discrepancy between the size of the particles observed in natural and experimental scrapie and the radiation data on the probable size of the transmissible agent could possibly be explained by postulating that incomplete viral particles have the capacity to produce abnormalities in the replication of neuronal cell membranes of the kind described in this report.

In sheep, particles were found solely within the neuronal lesions which are characteristic of natural scrapie and have not been reported in other neuropathological conditions. It should be added, however, that only a small minority of such neuronal lesions contained these aggregations of particles. A similar situation obtains in chick embryo cells infected by Semliki Forest virus or Sindbis virus, in which intravacuolar budding of membranes with no visible viral particles has been reported (*9*).

A. BIGNAMI

Department of Pathology, Stanford Medical Center, Stanford, California 94305

H. B. PARRY

Nuffield Institute for Medical Research, Oxford, OX3 9.DS, England

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Hyperoxaluria in L-Glyceric Aciduria: Possible Pathogenic Mechanism

Abstract. *The effect of hydroxypyruvate on synthesis of oxalate and glycolate from glyoxylate was studied in in vitro preparations from normal human erythrocytes and leukocytes, rat liver, and with purified lactate dehydrogenase from beef heart. In the presence of reduced nicotinamide adenine dinucleotide, hydroxypyruvate stimulated the oxidation of glyoxylate to oxalate and decreased the reduction of glyoxylate to glycolate. These findings may explain the hyperoxaluria seen in* L*-glyceric aciduria (type II primary hyperoxaluria).*

L-Glyceric aciduria (primary hyperoxaluria, type II) is a metabolic disease secondary to a genetic defect in D-glyceric dehydrogenase, an enzyme which catalyzes the reduction of hydroxypyruvate to D-glycerate in the degradative pathway of serine metabolism (Fig. 1) (*1*). It is presumed that hydroxypyruvate accumulates and is reduced by lactate dehydrogenase to L-glycerate, which is excreted in large amounts (300 to 600 mg per 24 hours) in the urine. There is an associated excessive urinary excretion of oxalate, which leads to the sole clinical manifestation of the disease, calcium oxalate nephrolithiasis. In contrast to primary hyperoxaluria type I, urinary glyoxylate and glycolate are normal or reduced rather than elevated (*2*). Previous studies from this laboratory demonstrated that [^{14}C]hydroxypyruvate was not incorporated into urinary oxalate and [1-^{14}C]-glyoxylate was not incorporated into urinary L-glycerate in a patient with L-glyceric aciduria (*1*). The link between the enzymatic defect and the resulting hyperoxaluria has therefore remained obscure. The following study was undertaken to examine the effect of hydroxypyruvate on the synthesis of oxalate and glycolate from glyoxylate as a possible explanation for the associated hyperoxaluria of L-glyceric aciduria.

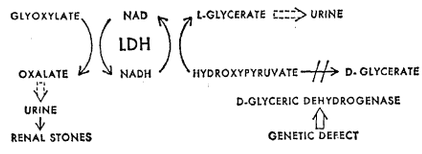

Fig. 1. Metabolic pathways of hydroxypyruvate and glyoxylate; *LDH*, lactate dehydrogenase.

The conversion of [1-^{14}C]glyoxylate to oxalate and glycolate was studied in hemolyzates of normal human erythrocytes, in normal human leukocytes disrupted by high-frequency sound, in homogenates of rat liver (100,000*g* supernatant), and in partially purified preparations of lactate dehydrogenase from beef heart and from rabbit muscle. Formation of oxalate was determined by decarboxylation with purified fungal oxalate decarboxylase (*3*) with trapping of the $^{14}CO_2$ in Hyamine for counting in a Tri-Carb liquid scintillation spectrometer (50 percent recovery for [^{14}C]oxalate). Carrier glycolate was added at the completion of the experiment, and the amount of glycolate synthesized from [^{14}C]glyoxylate was determined from its specific activity after purification by ion-exchange and paper-chromatographic procedures (*4*).

Hydroxypyruvate enhanced the synthesis of oxalate from [1-^{14}C]glyoxylate in human erythrocyte hemolyzate preparations (Fig. 2). At a concentration of hydroxypyruvate of 0.02*M* there was a fourfold stimulation of oxalate synthesis. At similar concentrations two other keto acids, *p*-hydroxyphenylpyruvate and α-ketoglutarate, had no effect on oxalate synthesis in this system. A similar stimulation of oxalate synthesis from glyoxylate by hydroxypyruvate was observed in human leukocytes disrupted by high-frequency sound and in preparations of rat liver.

Lactate dehydrogenase catalyzes the reduction of hydroxypyruvate to L-glycerate. It also catalyzes the linked oxidation of glyoxylate to oxalate and its reduction to glycolate (*5*). The effect of hydroxypyruvate on synthesis of oxalate and glycolate from [1-^{14}C]glyoxylate was therefore studied with preparations of lactate dehydrogenase from beef heart and rabbit muscle. In the presence of nicotinamide adenine dinucleotide (NAD) the rapid oxidation of glyoxylate to oxalate by lactate dehydrogenase is unaffected by hydroxypyruvate. In the presence of reduced nicotinamide adenine dinucleotide

(NADH), hydroxypyruvate (2.5×10^{-4} to $2 \times 10^{-3}M$) markedly stimulated the conversion of glyoxylate to oxalate and decreased the reduction of glyoxylate to glycolate (Fig. 3). This effect was observed equally with preparations of lactate dehydrogenase from beef heart and rabbit muscle. Pyruvate in comparable concentrations also stimulated oxalate synthesis but *p*-hydroxyphenylpyruvate had no effect. At concentrations of hydroxypyruvate greater than $5 \times 10^{-3}M$ a decrease in the stimulation of oxalate synthesis was observed.

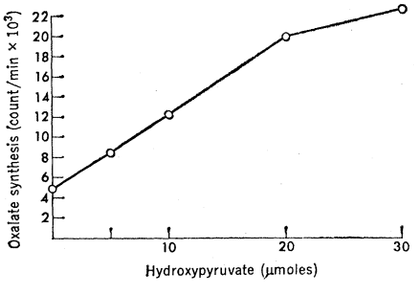

Fig. 2. Effect of various concentrations of hydroxypyruvate on synthesis of oxalate from glyoxylate in human erythrocyte-hemolyzate system. Reaction mixture contained 1.5 μmole of sodium [1-^{14}C]glyoxylate (254,000 count/min per micromole), 100 μmole of phosphate buffer (*p*H 7.4), and erythrocyte hemolyzate containing 20 mg of hemoglobin in a final volume of 1.0 ml. Mixture was incubated for 30 minutes at 37°C; reaction was stopped with addition of 0.1 ml of 1*M* citric acid, and decarboxylation of formed [^{14}C]oxalate was carried out by addition of purified fungal oxalate decarboxylase (*3*) with trapping of $^{14}CO_2$ in Hyamine for liquid scintillation counting.

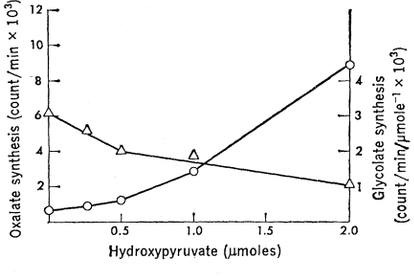

Fig. 3. Effect of various concentrations of hydroxypyruvate on synthesis of oxalate and glycolate from glyoxylate catalyzed by lactic dehydrogenase. Reaction mixture contained 0.75 μmole of sodium [1-^{14}C] glyoxylate (254,000 count/min per micromole), 100 μmole of phosphate buffer (*p*H 7.4), 0.5 μmole of NADH, 5 μg of beef heart lactate dehydrogenase type III (Sigma) in a final volume of 1.0 ml. The mixture was incubated for 30 minutes at 37°C. Oxalate was determined as described in Fig. 2. Glycolate synthesis was determined by addition of 20 μmole of carrier glycolate at the end of the incubation period followed by ion-exchange chromatography on Dowex-1-acetate, paper chromatography, and determination of radioactive glycolate with 2,7-dihydroxynaphthalene and liquid scintillation counting (*4*). ○, Oxalate synthesis; △, glycolate synthesis.

These data indicate that hydroxypyruvate is capable of stimulating the oxidation of glyoxylate to oxalate in a coupled reaction catalyzed by lactate dehydrogenase (LDH) in the presence of NADH, as reported for pyruvate (*6*). These observations offer an explanation for the increased synthesis and excretion of oxalate in L-glyceric aciduria, in which the defect in D-glyceric dehydrogenase presumably leads to the intracellular accumulation of hydroxypyruvate (Fig. 1). This effect of hydroxypyruvate apparently results from the increased oxidation of the LDH-NADH complex secondary to the reduction of hydroxypyruvate to L-glycerate. This shift in the ratio NAD:NADH may also explain the concomitant reduction in glycolate synthesis from [^{14}C]glyoxylate observed in these patients (*1*). The decrease in the stimulation of oxalate synthesis at higher concentrations of hydroxypyruvate may be related to competition of hydroxypyuvate with glyoxylate for catalytic binding sites on the enzyme.

This explanation for the increased oxalate synthesis found in L-glyceric aciduria represents a novel mechanism for the phenotypic expression of a human genetic disease. The recent description of a patient with chronic lactic acidosis associated with an altered reduction-oxidation state of several NAD:NADH-coupled reactions suggests a somewhat analogous but more generalized metabolic derangement, although no specific enzymatic defect was found (*7*). It can also be anticipated that the accumulation of other keto acids which are substrates for lactate dehydrogenase might secondarily result in hyperoxaluria and hypoglycolic aciduria. Such an additional patient has been found and this explanation is being pursued (*8*). Finally, this further emphasis of the important role of lactate dehydrogenase in oxalate synthesis in man suggests a possible site for the development of metabolic inhibitors. An effective inhibitor of oxalate synthesis would allow a rational approach to the treatment of primary hyperoxaluria, a serious and often fatal group of diseases.

HIBBARD E. WILLIAMS
LLOYD H. SMITH, JR.
Department of Medicine, University of California School of Medicine, San Francisco 94122

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Selective Stimulation of Allelic Expression: Effect of Antibodies to Allotypic Markers on Lymphoid Cells

Abstract. *Peripheral blood leukocytes from rabbits which were heterozygous* (b^5/b^9) *for markers on their immunoglobulin light chains were maintained in vitro for up to 24 hours in the presence or absence of antibody to b9. After culture they were transferred into lethally irradiated* b^4/b^4 *hosts. Recipients of cells exposed to antibodies to allotype markers showed a striking increase in concentration of circulating b9 molecules and number of b9 plasma cells in their spleens compared to control animals receiving untreated cells from the same donor. There was no appreciable difference between the two groups of recipients with respect to their content of b5 molecules and immunocytes.*

Allogeneic transfer of lymphoid cells into lethally irradiated recipient rabbits has been used to assess the potential of the donor cells to proliferate and differentiate into immunocytes (*1*). The donor or recipient origin of the antibody-synthesizing and secreting cells has been established by identification of the genetically determined allotypic markers on the κ light chains of their product immunoglobulins (*2*). Our assay for precursors of immunocytes in the