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16. Supported by PHS grants MH-17071 and MH-5999. We thank Dorothy Dembiec for her technical assistance.

24 February 1970; revised 30 October 1970 ■

Aggregations of 35-Nanometer Particles Associated with Neuronal Cytopathic Changes in Natural Scrapie

Abstract. *Neuronal vacuolation and intravacuolar budding of vesicles and cytoplasmic processes appear to be the most characteristic cellular lesion in natural scrapie, a chronic degenerative disease of the central nervous system of sheep which is transmissible by injection. Membrane-bound accumulations of the 35-nm particles are found in the cytoplasmic processes that project inside the vacuoles of the neuronal perikaryon. Such particles are present only in a small number of vacuolated neurons.*

Scrapie is a progressive degenerative disease of the central nervous system of sheep. It has been experimentally transmitted by injection to sheep (*1*), goats (*2*), and, more recently, to small laboratory animals (*3*) after long incubation periods. Vacuolation of neurons is a characteristic of the disease (*4*). In the present study, five sheep affected with scrapie and seven normal sheep were satisfactorily perfused with glutaraldehyde for electron microscopy.

Large membrane-bound vacuoles were found within neuronal cell bodies, dendrites, and axonal terminals in the medulla, pons, cerebellar cortex, supraoptic nucleus of the hypothalamus, paramammillary nucleus, and pyramidal layer of the hippocampus. Vacuolation of glial cells was not observed. Small invaginations indicative of pinocytosis were found along the vacuolar membrane, which suggests that it possessed some of the features of a plasma membrane.

The most striking feature was that of vesicles 100 to 150 nm in diameter, and of multiple cytoplasmic projections budding inside the vacuole (Fig. 1). In some vacuolated neurons these projections contained membrane-bound accumulations of round particles of uniform size, 35 nm in diameter (Fig. 2). Some of the particles displayed a dense central core. Others had an electron-lucent center (Fig. 2, inset). Particles of similar size have been observed in large amounts within the distended neuronal cell processes of the cerebral cortex of mice injected with experimental scrapie material (*5*). These particles lay free in the cytoplasm and had electron-lucent centers. They were not thought to represent the scrapie agent (*5*), mainly because of the discrepancy between the size of the particles and the size of the scrapie infectious agent as determined by radiation experiments. The scrapie

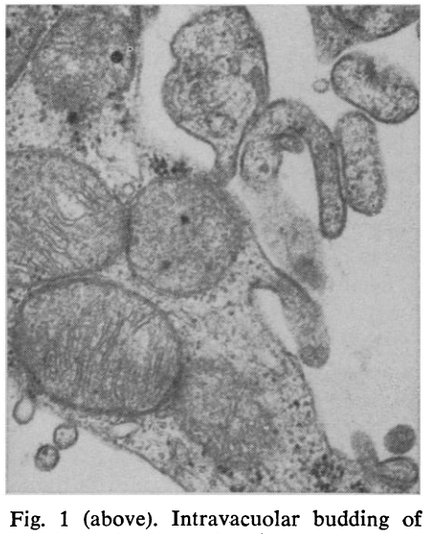

Fig. 1 (above). Intravacuolar budding of vesicles and of cytoplasmic processes in a pontine neuron (× 31,000).

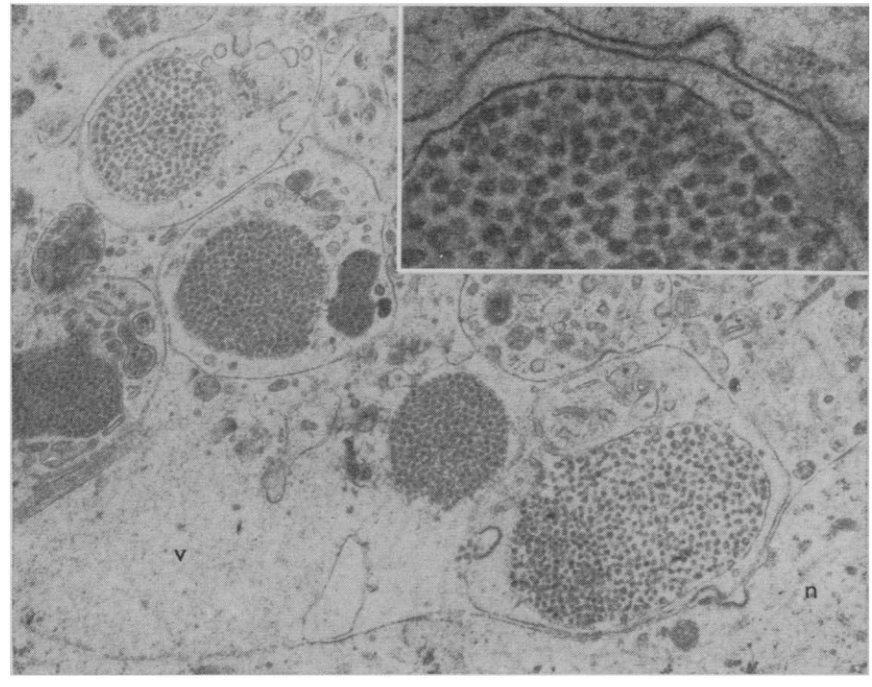

Fig. 2 (right). Membrane-bound collections of 35-nm particles within a vacuolated neuron (*n*) in the supraoptic nucleus of the hypothalamus. These collections are contained in cytoplasmic processes extending inside the vacuole (*v*) (× 21,000). (Inset) An electron dense core is recognizable in some of the particles (× 53,000).

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*).

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14 September 1970

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.

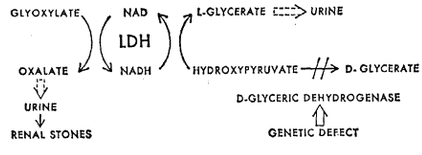

Fig. 1. Metabolic pathways of hydroxypyruvate and glyoxylate; *LDH*, lactate dehydrogenase.

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