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Histopathology of Hydrocephalus Resulting from a Deficiency of Vit. B_{12}.* (23646)

P. M. NEWBERNE† AND B. L. O'DELL

Department of Agricultural Chemistry, University of Missouri, Columbia

A high incidence of hydrocephalus and other congenital malformations occurs in infant rats from Vit. B_{12} deficient mothers(1). The cerebral aqueduct in the hydrocephalic brain is partially or completely occluded and a unique type of tall columnar cell is absent from the roof of the third ventricle and cerebral aqueduct(2). Brains from litter mates of hydrocephalic animals have aqueducts of abnormal size and shape although their gross appearance is normal.

This paper is an extension of previous studies made in our laboratories on the hydrocephalic brain in newborn rats from Vit. B_{12} deficient dams. The significant observations were that the ependymal lining and choroid plexus were deranged and contained excess lipide.

Methods. Weanling female rats of the Wistar strain were fed a Vit. B_{12} deficient diet, composed chiefly of soybean meal and glucose(1) until they reached a weight of 160-170 g. During the reproductive phase some of the animals were continued on this diet while control animals received the same diet supplemented with 30 μg of Vit. B_{12}/kg. Thirty-two Vit. B_{12} deficient females bore 896 offspring comprising 132 litters. Of this number 92, or about 10%, were hydrocephalic. This abnormality was detected at birth by sectioning the gross brain with a knife blade after fixation in Bouin's solution. In the normal brain there is no grossly detectable distension of the lateral ventricles. Ventricular distension in hydrocephalic brains varied from 1 to 2 mm in mild cases to 6 to 8 mm in severe cases. A higher percentage of mild cases was detected by this method than by the light transmission technic(3) and resulted in approximately doubling of the observed incidence. No hydrocephalus was demonstrated at birth among offspring of control dams. For routine observation 7 μ sections were cut and stained with hematoxylin and eosin. Brains used for fat studies were stained with osmic acid(4) and Herxheimer's fat stains(5). Brains from 34 hydrocephalic offspring, 35 litter mates and 28 controls were examined.

Results. In cases of gross dilatation of the lateral ventricles the cerebral tissue was reduced to the thinness of a membrane. Microscopically, the cortical layers were disrupted and in some areas of the lateral and third ventricles the thinned ependymal lining was folded outward. In other areas, notably in the third ventricle, the ependyma was absent and the brain tissue appeared areolar and

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† Public Health Service Research Fellow, Nat. Inst. of Neurological Diseases and Blindness.

showed distended spaces as though it were saturated with fluids. Detached and scattered cuboidal ependymal cells were morphologically similar to round cells. (Fig. 1, 2). In less severe cases of hydrocephalus the columnar type ependymal cells normally found in many areas had been replaced by cuboidal type cells (Fig. 3, 4). Ependymal cells and

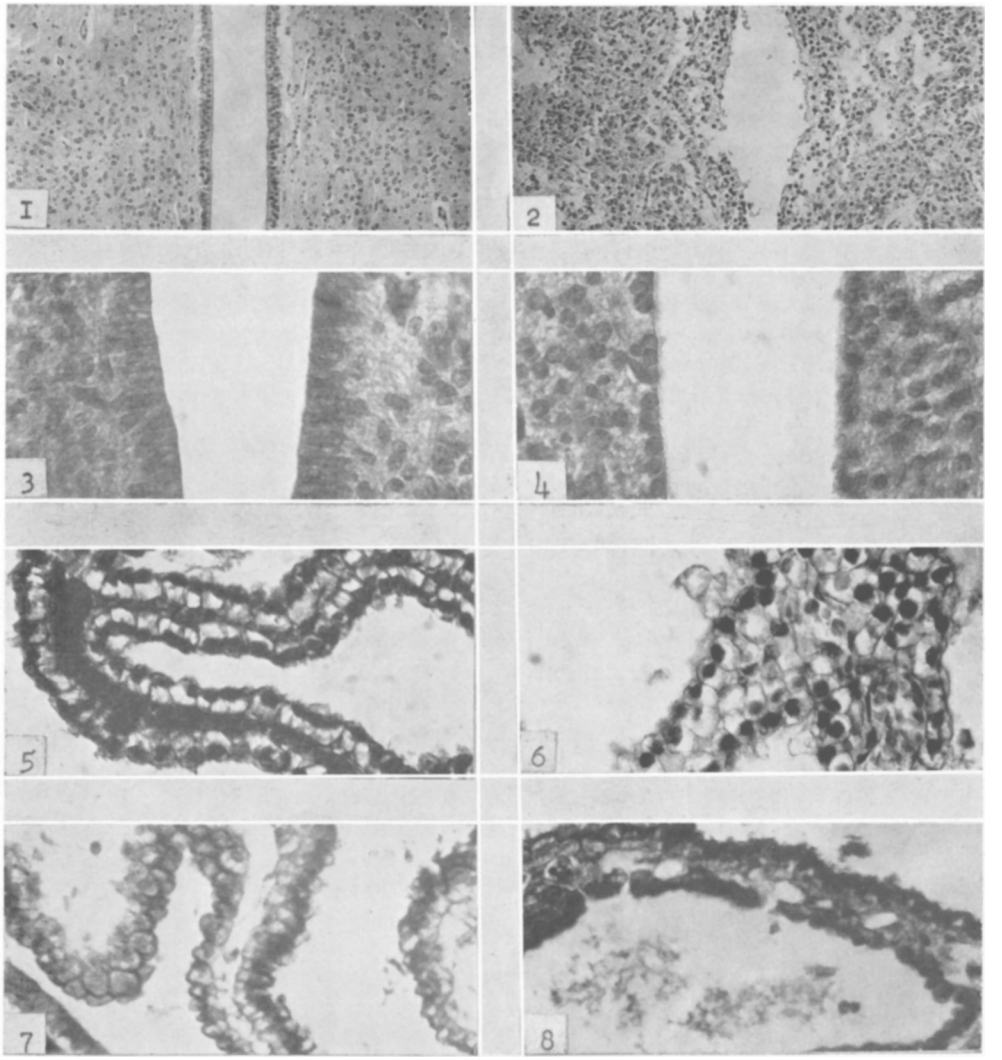

FIG. 1. Third ventricle from control rat showing normal ependymal lining. × 80.

FIG. 2. Third ventricle from experimental rat with areas of ependyma entirely missing. Edema and clumping of tissue gives appearance of increase in cells. This is an artefact. The cells in tissue adjacent to the lumen are detached and scattered cuboidal type cells from the ependymal lining. × 80.

FIG. 3. Third ventricle from control rat showing elongated columnar-type ependymal cell. × 320.

FIG. 4. Third ventricle from experimental rat with cuboidal type cell of the thin ependyma. × 320.

FIG. 5. Choroid plexus, lateral ventricle of control rat, with well defined cellular appearance. × 320.

FIG. 6. Choroid plexus, lateral ventricle of experimental rat, with dilatation and fragmentation of cells.

FIG. 7. Choroid plexus, lateral ventricle of control rat. Osmic acid fat stain. × 320.

FIG. 8. Choroid plexus, lateral ventricle of experimental rat, showing dilated cells filled with fat and fat stained debris in the cavity. Osmic acid stain. × 320.

surface debris stained darkly with osmic acid indicating the presence of excess lipide.

The cerebral aqueduct was occluded in 19 of the 34 hydrocephalic brains examined, but was stenotic and irregularly shaped in the other 15. Tall columnar cells normally present as an intact layer in the roof of the third ventricle and aqueduct were absent in most hydrocephalic brains although they were observed in some cases with completely occluded aqueducts.

The choroid plexus in hydrocephalic brains had lost its normal architecture. The cytoplasm in many cells was disrupted and granular and exhibited large vacuoles. The centrally located nuclei were pyknotic in many instances and varied in size and appearance. The cells appeared torn and fragmentation occurred into the ventricular cavity (Fig. 5, 6). Osmic acid stain showed that the cells were filled with lipide and that much of the debris was fatty in nature (Fig. 7, 8). Herxheimer's stain on frozen sections confirmed these observations.

There were mild glial changes around the lateral ventricle and less marked changes in the area of the third ventricle and aqueduct. The number of cells per unit area around the aqueduct was 15% higher in the experimental animal. The averages of 10 areas (0.74 x 0.22 mm) of the constant depth in 4 experimental and 4 control brains were 73 and 63, respectively.

Brains from litter mates of hydrocephalic animals showed no distension of ventricles, but the aqueducts were irregular in shape. The choroid plexus and ependymal cells were affected in the same manner as those of hydrocephalic animals but to a lesser degree. The tall columnar-type cells normally found in the roof of the third ventricle and aqueduct were always present in non-hydrocephalic litter mates.

Discussion. Previous observations(2) as well as those reported here show that hydrocephalus in the Vit. B_{12} deficient newborn rat is accompanied by stenosis or complete occlusion of the cerebral aqueduct. Whether or not the latter is the cause of ventricular dilatation or is simply associated with it cannot be determined in the light of present knowledge of origin and absorption of cerebrospinal fluid. Evidence exists to show that the cerebrospinal fluid is formed by the choroid plexus although the mechanism is not clear(6). Hassin(7) opposes the theory that the plexuses are the site of formation of cerebrospinal fluid and holds that they act as an absorbing and purifying apparatus. Hoehn (8) showed by means of artificially induced hydrocephalus that fluids in the plexus flow in both directions and that resorption of the fluid by blood vessels of the choroid plexus takes place. Granules of iron pigment are frequently found in the choroid epithelium in cases where ventricular hemorrhage has occurred. This may be regarded as evidence of absorptive activity on the part of the plexus but does not preclude concomitant secretory activity.

The presence of large quantities of fat in the ependymal lining cells and choroid plexus, as observed in this study might have a marked effect on the rate of absorption of cerebrospinal fluid. The type of lipide present could indeed affect absorption or elaboration of fluid(9). Cerebrospinal fluid pressure in hydrocephalus due to Vit. B_{12} deficiency may rise to 350 mm of water(3). In order for pressure of this magnitude to develop there must be an active secretion and it appears that hydrocephalus observed in Vit. B_{12} deficient rats is the result of a decreased rate of absorption of cerebrospinal fluid. This may result from a reduction of flow through the cerebral aqueduct or impaired absorption due to fat deposition in absorptive tissues.

It has been suggested(2) that the intact layer of tall columnar cells in the roof of the third ventricle and aqueduct as seen in control animals has a secretory function and helps maintain a patent aqueduct. Although absent in many hydrocephalic brains, our results show that these cells are present above some of the completely occluded aqueducts. These cells may have been non-functional but it seems unlikely that their absence is the primary defect in this type of hydrocephalus. Globus and Bergman(10) suggested that gliosis is a common cause of cerebral aqueduct stenosis in human infants. The mild glial increase observed in this study could hardly

account for complete closure of the aqueduct. As pointed out by Russell(11), gliosis is evoked in subependymal tissues by distension of the ventricles and destruction of the ependymal epithelium. This presumably arises as a reaction to pressure and tends to counteract pressure of fluid from within the ventricle. Bruemmer *et al.*(12) found a 15% increase in the number of nuclei/g of tissue in brain homogenates from Vit. B_{12} deficient offspring. There was no difference in the amount of deoxyribonucleic acid per nucleus, but the amount of pentose-nucleic acid per cell was decreased. It was concluded that the average brain cell in the deficient animal is smaller than normal, resulting in an increased number of cells per unit volume.

Summary. Female rats deficient in Vit. B_{12} produced offspring with a high incidence of hydrocephalus. The cerebral cortex of hydrocephalic brains was decreased in thickness and in some areas the ependyma was missing. Ependymal and choroid cellular morphology was deranged and the cells had accumulated lipide. Tall columnar-type cells normally present as an intact layer in the roof of the third ventricle and aqueduct of control rats were occasionally found in hydrocephalic brains with occluded aqueducts.

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Nicotinic Acid—Tryptophan Metabolism in Certain Diseases.* (23647)

SACHCHIDANANDA BANERJEE AND P. S. AGARWAL
Department of Physiology, Presidency College, Calcutta, India

Biosynthesis and metabolism of nicotinic acid has been extensively studied(1-6) in animals and in normal humans but comparatively little work has been done on this subject in persons suffering from diseases. Conflicting claims have been made regarding the principal site of synthesis of nicotinic acid. Liver(7-11), intestinal bacteria(12-16), and body tissues(17-21) have been suggested as the major site of this vitamin formation. The present communication deals with studies on the urinary excretions of nicotinic acid and amide, quinolinic acid, N'-methylnicotinamide (N'MN), 6-pyridone, tryptophan, kynurenin, anthranilic acid and 3-hydroxyanthranilic acid in patients suffering from typhoid fever, cholera, small pox, liver cirrhosis, and infective hepatitis before and after feeding of tryptophan with a view to study the metabolism of nicotinic acid and its site of synthesis from tryptophan. For the sake of comparison similar studies were made on normal men also.

Methods. Ten male patients suffering from each disease who were admitted in the N. R. Sircar Medical College Hospital, Calcutta

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