The pathogenesis of hydrocephalus in newborn rats deficient in vitamin B$_{12}$

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Richardson & Hogan (1946) produced hydrocephalus in rats by feeding a purified casein diet containing all the vitamins available at that time. Almost 2% of the young from females fed the experimental diet were hydrocephalic; female rats fed the same diet supplemented with liver extract gave birth to normal young. It is now thought that this diet was marginally deficient in vitamin B$_{12}$ (Newberne & O'Dell, 1961). Animals which showed no gross evidence of lesions in the central nervous system had reduced maze-learning abilities as compared to animals on control diets (Whitley, O'Dell & Hogan, 1951).

The frequency in occurrence of hydrocephalus was greatly increased by changing the source of protein in the diet from casein to soybean oil meal, and also by adding X-methyl folic acid. Vegetable proteins, such as soybean oil meal, are known to have a lower content of vitamin B$_{12}$ than does the protein from milk. The use of the folic acid antagonist produced spina bifida, cranium bifida, anophthalmia, microphthalmia, cleft palate, short mandible, and edema in addition to hydrocephalus (O'Dell, Whitley & Hogan, 1951). The addition of vitamin B$_{12}$ to the antagonist-containing diet prevented the occurrence of hydrocephalus. Hydrocephalus in offspring littered to vitamin B$_{12}$-depleted dams was not prevented by the addition of folic acid to the ration. Giroud, Lefebvres & Dupuis (1952) used 5% succinylsulfathiazole in a purified diet to produce a folic-acid deficiency in mother rats, and hydrocephalus was observed in many of the young from these females.

O'Dell, Whitley & Hogan (1948) were also able to show that if female rats are depleted of vitamin B$_{12}$ they produce litters with a high incidence of hydrocephalus. This was true even though the diet was supplemented with folic acid and did not contain a folic acid antagonist. The parenteral administration of

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crystalline vitamin $B_{12}$ to pregnant rats before the twelfth day of gestation seemed to prevent the development of hydrocephalus.

Overholser, Whitley, O'Dell & Hogan (1954) concluded that congenital hydrocephalus due to deficiencies of vitamin $B_{12}$ or folic acid was caused by occlusion of the cerebral aqueduct in the embryo between the sixteenth and eighteenth day of gestation. They found the closed portion of the aqueduct open before birth but stated that it remained abnormal in size and shape. The occlusion of the cerebral aqueduct was associated with the absence of a specialized group of columnar ependymal cells (the subcommissural organ) in the roof of the cerebral aqueduct and the posterior part of the roof of the third ventricle. Newberne & O'Dell (1958) confirmed that hydrocephalus in vitamin $B_{12}$ deficiency is accompanied by stenosis or complete occlusion of the aqueduct. Their work also showed that the columnar cells of the subcommissural organ were present but often abnormal. Bruemmer, O'Dell & Hogan (1955) found a 15% increase in the number of nuclei per gram of tissue in brain homogenates from vitamin $B_{12}$-deficient offspring. There was no difference in the amount of deoxyribonucleic acid (DNA) per nucleus, but the amount of ribonucleic acid (RNA) per cell was decreased. It was concluded that the average brain cell in deficient animals was smaller than normal, resulting in an increased number of cells per unit volume.

The subcommissural organ was shown by Wislocki & Leduc (1952, 1954) to be a specialized area of tall columnar ependymal cells. These cells are secretory in nature and elaborate a substance which clings to the luminal surface of the ependymal cells and accumulates within the lumen of the aqueduct where it condenses to form Reissner's fiber. Newberne (1962) demonstrated that in rats the cells of the subcommissural organ and certain periaqueductal cells contain large quantities of substances which are histochemically similar to that described previously by Wislocki & Leduc. In vitamin $B_{12}$-deficient hydrocephalic newborn rats only insignificant amounts of these substances were noted, but moderate quantities were seen in nonhydrocephalic littermates.

Stempak (1965) produced hydrocephalus in newborn rats by giving the dams a folic acid-free diet with added folic acid antagonists (X-methyl folic acid and 9-methyl pteroylglutamic acid) for a 48 h period beginning on the eighth and ending on the tenth day of gestation. Occlusion or extreme stenosis of the aqueduct of Sylvius was demonstrated in all hydrocephalic animals. The first indications of hydrocephalus were present on the eighteenth day of gestation. No distention of the ventricles was seen in 17-day-old fetuses; however, aqueducts with varying degrees of stenosis were demonstrated. Stenosis of the aqueduct was observed in fetuses as early as the sixteenth day of age.

Previous studies have indicated that hydrocephalus might be associated with an abnormality of the columnar cells of the cerebral aqueduct. Alterations of the fluid balance have also been implicated as being related to hydrocephalus in vitamin $B_{12}$-deficient offspring (Newberne, 1962). The present studies were
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MATERIALS AND METHODS

Vitamin B_{12} depletion of adult animals was accomplished by raising the animals from weaning on a ration deficient in vitamin B_{12} and low in cobalt. The diets used in this experiment have been shown to result in low liver levels of vitamin B_{12} in deficient animals when compared to controls. The basal ration consisted of the following ingredients (as percentages): alpha protein, 30; sucrose, 44.7; salts, 5; vitamin mix, 10; cottonseed oil, 10; and DL-methionine, 0.3. The vitamin mix contained (in mg/100 g of sucrose carrier): thiamine · HCl, 16; pyridoxine · HCl, 16; riboflavin, 16; Ca pantothenate, 40; nicotinamide, 12; folic acid, 5; inositol, 250; vitamins A and D, 70; vitamin E, 450; and menadione, 10. One gram of 'vitamin A and D' is equivalent to 325000 u.s.p. units of vitamin A and 32500 u.s.p. units of vitamin D_{2}. One gram of 'vitamin E' is equivalent to 259 mg of vitamin E as DL-α-tocopherol acetate. It has been shown in our laboratory that these diets do not appear to affect growth and maturation of the female rat; however, they do have a detrimental effect on the reproductive performance including fetal resorption, pre-natal mortality, and birth weight of offspring. Albino rats of the Sprague-Dawley, Caesarean-Derived strain were obtained from Charles River Breeding Farm. Females were raised in elevated, screen-bottom cages from weaning to a breeding age of 3 months or until body weight reached 160–180 g. Food and water were supplied ad libitum. During the growth period, the basal diet was supplemented with 0.1 % of choline chloride. At adulthood, females were divided into four different groups and fed diets as follows: (1) basal diet; (2) basal diet with 0.1 % of choline chloride added; (3) basal diet with 10 mg of X-methyl folic acid per kilogram of diet and 0.1 % of choline chloride added; and (4) basal diet with 0.1 % of choline chloride and 50 μg of vitamin B_{12} per kilogram of diet added. After the females had been on the experimental diets for 3 weeks, normal males were added to their cages. Females were allowed to litter within their cages, and young were collected soon after birth, or were allowed to fall through the mesh floor and were collected from the paper beneath. For some studies young were delivered by Caesarean section on various days of gestation using the day sperm were found in a vaginal smear as day zero. Experimental animals used in the biochemical studies were newborns from dams raised on the choline-supplemented or vitamin B_{12}- and choline-supplemented basal diets; newborn animals from all four groups were used in the morphologic studies.

Measurements of urine and blood electrolytes and serum proteins were chosen as indices of fluid balance in the newborn animals. Blood was collected from individual newborn animals in microhematocrit tubes, centrifuged for 10 min, and values recorded. Cellular components were separated from the serum by
carefully breaking the hematocrit tube at the appropriate location. Urine samples were collected from the newborn animals by clamping the urethral opening with a pair of mosquito forceps. The urinary bladder was then dissected free, blotted with tissue paper, and the urine sample allowed to flow into capillary tubes by releasing the forceps.

The specific gravity of the urine and the total protein content of the serum were calculated from the refractive indices of these materials. The refractive index was measured using a Goldberg refractometer (TS meter, American Optical Company). Conversions were made by means of tables calibrated for the instrument. The sodium and potassium content of the urine and serum was determined using a Coleman Flame Photometer. The method, using micro-quantities of sample, was that outlined for the instrument. A standard was prepared using a commercial serum of known electrolyte composition. A Beckman Model R paper-electrophoresis system was used for determining the percentage of serum proteins found in deficient and control animals. The methods recommended by the manufacturers were used in all cases. Buffer B₂ (ionic strength, 0.075; pH, 8.6), Beckman Instruments, Inc., was used in separating the serum fractions. The power supply was operated at a constant current of 2.5 mA for a period of 16 h.

Fetuses and newborn animals were decapitated, and the heads were fixed in formalin, Bouin's or Zenker's solutions. Only brains from those deficient litters in which hydrocephalic animals could be detected grossly were examined histologically and compared with controls. Tissues were prepared for processing by trimming the fixed head with a razor blade and then removing the brain. Cuts were made sagittally just lateral to the midline, or coronally anterior to the pineal and posteriorly through the fourth ventricle. Tissues were paraffin-embedded by routine Technicon procedures and sections were cut at 7 μ. Serial sections through the cerebral aqueducts were stained using hematoxylin and eosin, luxol fast blue-cresyl violet, or alcian blue-PAS techniques (Armed Forces Institute of Pathology, 1960).

**RESULTS**

An $F$ value was computed in testing the null hypothesis that there were no differences between vitamin $B_{12}$-deficient and control groups. The statistical evaluation of the biochemical data is presented in an analysis of variance table using a hierarchal classification. This system consists of a unique order of criteria, each criterion being applicable within all categories of the preceding criterion. Thus, the data were classified in terms of vitamin $B_{12}$-deficient or control dams, litters within dams, or neonatal offspring within litters. The experimental error used in judging the significance between deficient and control groups consisted of two sources of variation: variation among litters of newborn animals, and variation among individual offspring within the litter. Tests were made to determine if the variation among litters was of a different order of
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magnitude than among offspring. When litters were found to contain significant variation, in addition to that of the offspring, the mean square for litters of newborn animals was used as the error term to test for differences between deficient and control groups.

During the course of analysis of the electrolyte content of serum and urine, it was necessary to make up several reference standards. Since it was impossible to make the standards exactly the same each time, individual values from each litter were analysed according to the reference standard used. A difference was noted in the standard solutions, but the treatment/standard-solution interaction was not significant. The levels of serum and urine sodium were found to be higher in newborn animals from deficient mothers (Table 1). No large difference was noted in the serum potassium levels. The quantity of potassium was frequently greater than could be measured by the method available, and the amount of urine available did not permit duplication; therefore, statistical analysis could not be performed.

Table 1. Biochemical data—mean values

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Vitamin B₁₂-deficient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum sodium</td>
<td>145 m-equiv./l.</td>
<td>137 m-equiv./l.</td>
</tr>
<tr>
<td>Serum potassium</td>
<td>6-8 m-equiv./l.</td>
<td>6-1 m-equiv./l.</td>
</tr>
<tr>
<td>Urine sodium</td>
<td>134 m-equiv./l.</td>
<td>119 m-equiv./l.</td>
</tr>
<tr>
<td>Urine potassium</td>
<td>&lt; 10 m-equiv./l.</td>
<td>&lt; 10 m-equiv./l.</td>
</tr>
<tr>
<td>Urine specific gravity</td>
<td>1-010</td>
<td>1-010</td>
</tr>
<tr>
<td>Packed cell volume</td>
<td>35 %</td>
<td>37 %</td>
</tr>
<tr>
<td>Quantity of serum protein</td>
<td>2-5 g/100 ml</td>
<td>3-1 g/100 ml</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>66-5 %</td>
<td>67-7 %</td>
</tr>
<tr>
<td>Serum alpha globulin</td>
<td>16-6 %</td>
<td>15-3 %</td>
</tr>
<tr>
<td>Serum beta globulin</td>
<td>17-0 %</td>
<td>16-6 %</td>
</tr>
</tbody>
</table>

The packed cell volumes in offspring from the two groups were analysed using Student's $t$ test and were not found to differ. The specific gravity of the urine from control and deficient groups was the same. Statistical analysis showed that the serum protein, on the average, was higher in those newborn animals whose mothers were receiving vitamin B₁₂ supplementation. Quantitative differences in serum concentration of total protein could be detected, but no differences in the percentage albumin or alpha or beta globulins were seen. Mean values for the deficient and control groups are shown in Table 1, and the analysis of variance is presented in Tables 2 and 3.

Necropsy examination of the neonatal animals occasionally revealed a wide variation in the dilatation of the lateral ventricles of the brain. Animals with greatly dilated ventricles had grossly distorted anatomical relationships between parts of the brain. Rarely would an animal have only slightly dilated ventricles, and in no case did there appear to be an external hydrocephalus. The fourth
Table 2. Summary of analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>D.F.</th>
<th>M.S.</th>
<th>P</th>
<th>D.F.</th>
<th>M.S.</th>
<th>P</th>
<th>D.F.</th>
<th>M.S.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among experimental treatment groups (A)</td>
<td>1</td>
<td>957.60</td>
<td>&lt;0.005</td>
<td>1</td>
<td>7.36</td>
<td>&lt;0.25</td>
<td>1</td>
<td>3725.81</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Among standard solutions (B)</td>
<td>2</td>
<td>760.35</td>
<td>&lt;0.005</td>
<td>2</td>
<td>19.62</td>
<td>&lt;0.025</td>
<td>2</td>
<td>1417.70</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Treatment group standard interaction, A x B</td>
<td>2</td>
<td>180.79</td>
<td>&lt;0.10</td>
<td>2</td>
<td>2.84</td>
<td>NS</td>
<td>2</td>
<td>642.59</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Among litters of newborn animals (C) in A B</td>
<td>21</td>
<td>54.59</td>
<td>&lt;0.005</td>
<td>21</td>
<td>4.72</td>
<td>&lt;0.005</td>
<td>21</td>
<td>313.75</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Among offspring (D) in C in A B</td>
<td>40</td>
<td>16.39</td>
<td></td>
<td>40</td>
<td>0.75</td>
<td></td>
<td>40</td>
<td>90.25</td>
<td></td>
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</table>

Table 3. Summary of analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Quantity of serum protein</th>
<th>Serum albumin (%)</th>
<th>Alpha globulin (%)</th>
<th>Beta globulin (%)</th>
<th>Urine specific gravity (M.S. = \times 10^{-6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.F.</td>
<td>M.S.</td>
<td>P</td>
<td>D.F.</td>
<td>M.S.</td>
<td>P</td>
</tr>
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<td>---------------------------------------------</td>
</tr>
<tr>
<td>Among experimental treatment groups (A)</td>
<td>1 4.70 &lt;0.005</td>
<td>1 33.74 NS</td>
<td>1 41.57 &lt;0.25</td>
<td>1 0.24 NS</td>
<td>1 2.513 NS</td>
</tr>
<tr>
<td>Among litters of newborn animals (B) within groups (A)</td>
<td>15 0.60 &lt;0.005</td>
<td>28 105.03 &lt;0.01</td>
<td>28 60.32 &lt;0.005</td>
<td>28 29.51 0.05</td>
<td>10 24.52 &gt;0.25</td>
</tr>
<tr>
<td>Among offspring (C) within litter (B)</td>
<td>44 0.10 —</td>
<td>71 48.19 —</td>
<td>71 20.58 —</td>
<td>71 18.01 —</td>
<td>34 8.381 —</td>
</tr>
</tbody>
</table>
Fig. 1. Sagittal mid-line section through a normal cerebral aqueduct of an embryo. This animal was a litter-mate to the hydrocephalic animal shown below. The pineal gland can be seen in upper right of photograph. Note heavy staining in the tall columnar cells (subcommissural organ) at the entrance of the cerebral aqueduct. PAS stain, ×117.

Fig. 2. Sagittal section through the aqueduct of a hydrocephalic embryo. The section is just lateral to the mid-line. The pineal gland (1) has not formed properly. A rudimentary subcommissural organ can be seen (2). Serial sections showed the entrance of the cerebral aqueduct (f) to have a more ventral position than is normal. Compression of the aqueduct is evident. Luxol fast blue-cresyl violet, ×117.
Series of coronal sections, proceeding from anterior to posterior, through the mid-brain of a hydrocephalic animal. The roof of the third ventricle is not greatly dilated (figs. 3, 4). The entrance of the aqueduct into the third ventricle appears normal, and the anterior portion of the subcommissural organ is normal (fig. 5). As the aqueduct proceeds distally, the columnar cells disappear (fig. 6), and the aqueduct becomes stenotic (figs. 7, 8). Luxol fast blue-cresyl violet, ×51.
Coronal sections, proceeding from anterior to posterior, through the mid-brain of a hydrocephalic neonatal rat. The roof of the third ventricle is cystic, and the anterior portion of the aqueduct and the subcommissural organ can be seen. The aqueduct is abnormal in shape (fig. 9). The cells of the subcommissural organ disappear in more distal portions (fig. 10). Such areas may contain fine droplets of PAS-positive material. The aqueduct joins the dilated infundibular portion of the third ventricle (figs. 11, 12). As the dorsal columnar cells reappear, the aqueduct becomes normal (figs. 13, 14). A higher-power view of the dorsal columnar cells is shown (inset, fig. 13). PAS stain, ×45. Luxol fast blue-cresyl violet, ×45.

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Series of coronal sections, proceeding from anterior to posterior, through the brain stem of a hydrocephalic animal. The subcommissural organ and the cerebral aqueduct were absent from the brain stem in the area ventral to the pineal gland. The beginning of the aqueduct can be seen as a blind tube (figs. 15, 16). The columnar cells of the blind tube are shown in more detail (fig. 17). As the aqueduct continues posteriorly, it joins with the dilated infundibular portion of the third ventricle (fig. 18). The columnar cells and the aqueduct become normal in more posterior portions (figs. 19, 20). Luxol fast blue-cresyl violet, ×45.

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ventricle appeared normal, and herniation of the cerebellum through the foramen magnum could not be detected.

Hydrocephalus which occurred in newborn animals whose mothers were receiving a diet deficient only in vitamin B₁₂ was compared microscopically to that which appeared in offspring whose dams were on the other vitamin B₁₂-deficient rations. Since all of the hydrocephalic animals showed some abnormality of the cerebral aqueduct, it was concluded that the developmental process was similar in all three vitamin B₁₂-deficient groups. The general pattern of alterations was similar, but there was some variation between hydrocephalic animals within any one group.

Studies of the ventricular system in normal rat embryos showed that the subcommissural organ became clearly recognizable microscopically between the fifteenth and sixteenth day of gestation. At this time, several areas of the ventricular system had tall columnar cells which contained PAS-positive material. Hydrocephalus was demonstrated as early as the eighteenth day of gestation; however, studies were not performed to determine when aqueductal stenosis could first be detected. Normal development of the pineal gland was delayed in some hydrocephalic embryos.

Aqueductal stenosis was a routine finding in hydrocephalic animals from all three groups. Abnormal embryologic development of the subcommissural organ or the columnar cells of the roof of the aqueduct was also seen (Plate 1, figs. 1, 2). The course of the cerebral aqueduct was altered in many hydrocephalic brains, and some alterations in structural relationships of the neural tissue were considered to be secondary to dilatation of the lateral and third ventricles. Compression of the aqueduct because of intracranial pressure could contribute significantly to aqueductal stenosis; however, the abnormal course of the cerebral aqueduct from the third to the fourth ventricle and the absence of a subcommissural organ indicated a malformation of the aqueduct.

When the subcommissural organ was absent or when only the most anterior portion appeared normal, the cerebral aqueduct often arose in the third ventricle in a ventral position in contrast to the normal dorsal location found in non-hydrocephalic animals. Careful study of many hydrocephalic brains showed a relationship between presence of columnar cells of the subcommissural organ and patency of the anterior aqueduct; it is unclear, however, whether this relationship was a cause or an effect of the process which led to aqueductal occlusion. Secretion of cerebrospinal fluid caused pressure with dilation of the third ventricle, and communication was established between the infundibular portion of the third ventricle and the cerebral aqueduct.

The columnar cells of the most anterior portion of the subcommissural organ were sometimes normal, but they disappeared as the aqueduct proceeded distally (Plate 2, figs. 6–8). The columnar cells would reappear as the aqueduct continued posteriorly and became normal (Plate 3, figs. 13, 14). Some brains showed no evidence of a subcommissural organ, and, in such cases, the cerebral
aqueduct joined the floor of the dilated third ventricle. Columnar cells were present in the roof of the distal portion of the anterior aqueduct although the subcommissural organ was absent. Often, when the subcommissural organ was not recognizable, collections of immature cells with vesicular nuclei and hair-like cytoplasmic processes were found in its normal locus. A fine, dusty sprinkling of PAS-positive droplets was scattered among the foci of nuclei. Such areas were considered to be aplastic cells of the subcommissural organ.

The severity and degree of ventricular dilatation of the hydrocephalic animals was not necessarily correlated with degree of stenosis of the aqueduct. Animals with patent aqueducts had greatly dilated ventricles and cystic dilatation of the third ventricle (Plate 5). Evagination of the choroid plexus was often observed in such cases.

Eighteen-day embryos taken by caesarean section from dams fed the basal ration (vitamin B₁₂- and choline-deficient) showed severe malformation of brain development. The microscopic topographic relationships between various neural structures were misplaced. Both hydrocephalic and non-hydrocephalic littermates were observed, and absence of a complete aqueduct joining the third and fourth ventricles was seen in one non-hydrocephalic embryo. Abnormalities in the formation of the pineal gland and choroid plexus were noted, and aplasia of columnar ependymal cells was evident. Nests of cells which had either a central lumen or which formed rosettes were seen dorsal to the cerebral aqueduct and were considered to be accessory aqueducts or abortive attempts to form aqueducts (Plate 6).

**DISCUSSION**

Studies of some of the accepted parameters of fluid balance demonstrated a highly significant difference between newborn deficient and control animals in the quantities of serum protein and serum and urine sodium. The mean value for serum sodium in the deficient group was found to be eight m-equiv./l. higher than that of the control animals. However, this difference is of doubtful physiological importance. Since the levels of serum sodium were found to be high and those of serum protein low, the two substances would have opposing osmotic effects. The evidence does not warrant the conclusion that the congenital hydrocephalus was caused by an alteration in fluid balance.

Previous studies have indicated that hydrocephalus might be associated with an abnormal development of columnar cells in the roof of the cerebral aqueduct. This malformation was thought to cause stenosis of the aqueduct (Overholser et al. 1954). In some instances, however, a patent aqueduct was found, and in these cases the subcommissural organ was present (Newberne & O'Dell, 1959). In the present study, serial sections were made of the cerebral aqueduct of hydrocephalic brains, showing that there was usually a malformation of the subcomissural organ and stenosis of the cerebral aqueduct. In some hydrocephalic brains the greater portion of the subcommissural organ was normal;
Fig. 21. A coronal section through the brain of a normal animal. The third ventricle can be seen with its choroid plexus. Luxol fast blue-cresyl violet, × 102.

Fig. 22. A similar section from a hydrocephalic animal. As hydrocephalus becomes severe, the roof of the third ventricle becomes cystic, and the villi of the choroid plexus are evaginated by the pressure. Luxol fast blue-cresyl violet, × 102.

Fig. 23. A similar section taken from a severe hydrocephalic animal. The roof of the third ventricle is completely dilated, and evagination of the choroid plexus is complete. Numerous blood vessels surround the cystic roof. Luxol fast blue-cresyl violet, × 102.
in all cases, however, columnar cells in more distal portions of the aqueduct were absent. An abnormal aqueduct was seen in all instances.

The question has arisen whether increased secretion of cerebrospinal fluid could have caused an increase in ventricular fluid, dilatation of the lateral ventricles, and a secondary ductal stenosis. Interpretations of microscopic findings from the present study have led to the conclusion that stenosis of the aqueduct was not secondary to compression of the brain stem by fluid-filled ventricles. Abnormality in the formation of the cerebral aqueduct was probably the primary lesion, and this developmental defect resulted in hydrocephalus. These conclusions were based on the fact that the cerebral aqueduct was abnormal in all cases of hydrocephalus observed. The dorsal columnar cells of the aqueduct were absent in those areas where the aqueduct was abnormal. When the subcommissural organ was completely absent, the aqueduct did not enter the third ventricle in its normal dorsal position. The more distal portions of the anterior aqueduct were always abnormal, even when the major portion of the subcommissural organ was found to be normal in a hydrocephalic animal. The aqueduct appeared patent in some cases, and this was thought to be due to a secondary opening of the aqueduct. The following observations gave support to this conclusion: (1) patent aqueducts were always abnormal in either shape or interventricular route, (2) in some instances the aqueduct joined the third ventricle via the dilated infundibular portion, and (3) animals with patent aqueducts had large cystic third ventricles indicating an exceedingly high cerebrospinal fluid pressure.

PLATE 6

Fig. 24. A sagittal section through the brain of a hydrocephalic, 18-day embryo. The cerebral aqueduct (1) as well as accessory aqueductal structures (†) can be seen. Hematoxylin and eosin, ×158.

Fig. 25. A sagittal section through the same brain as shown in fig. 9. The dilated lateral ventricle can be seen at the right of the photograph. The pineal gland has only a rudimentary structure (†). Hematoxylin and eosin, ×236.

Figs. 26, 27. Higher magnification of accessory aqueductal structures shown in fig. 9. Ependymal cell nests have either a central lumen (26) or form rosettes (27) (†). Hematoxylin and eosin. ×1580.

Fig. 28. A sagittal section lateral to the mid-line through the brain of a non-hydrocephalic, 18-day embryo. This animal was a litter-mate to the animal shown in fig. 9. Severe malformations of the brain and cerebral aqueduct are evident. The third ventricle is divided into two compartments by rudimentary choroid appendages, and the abnormal cerebral aqueduct proceeds posteriorly just beneath the surface of the brain. Hematoxylin and eosin, ×63.

Fig. 29. Higher magnification of ependymal cells, choroid plexus, and cerebral aqueduct shown in fig. 28. Hematoxylin and eosin, ×1580.

Fig. 30. A mid-line, sagittal section through the brain shown in fig. 28. Serial sections showed that the cerebral aqueduct (†) failed to join the third (1) and fourth (2) ventricles. Hematoxylin and eosin, ×63.

Fig. 31. Higher magnification of the pineal gland and rudimentary choroid plexus shown in fig. 30. Hematoxylin and eosin, ×630.
Studies indicate that congenital hydrocephalus produced by vitamin B$_{12}$ deficiency or folic acid antagonists have a similar pathogenesis. Recent observations by Stempak (1965) that aqueductal stenosis could be detected as early as the sixteenth day and that ventricular dilatation was not detected until the eighteenth day (Overholster et al. 1954; Stempak, 1965) indicated that aqueductal stenosis preceded the formation of increased amounts of cerebrospinal fluid. Apparently the ventricles do not become distended until there is an active secretion of cerebrospinal fluid. This point is further substantiated by the finding of an incomplete aqueduct joining the third and fourth ventricles in a non-hydrocephalic 18-day-old embryo; this animal also had a rudimentary choroid plexus. Aplasia of the subcommissural organ and columnar ependymal cells, abnormalities in the formation of the pineal gland and roof of the third ventricle, and formation of accessory aqueductal structures indicate that the basic defect is concerned with the multiplication, migration, or maturation of the primitive neural elements.

**SUMMARY**

Findings indicate that the etiology is similar in hydrocephalus produced by vitamin B$_{12}$-deficient diets with and without added X-methyl folic acid. Observations tend to support the concept that hydrocephalus is caused by stenosis of the cerebral aqueduct associated with aplasia of the subcommissural organ and malformation of other neural structures. Biochemical studies showed no statistically significant difference between deficient and control groups in the packed cell volume of blood, specific gravity of urine, or quantity of serum potassium. Serum and urinary sodium were found to be greater in deficient animals, but the amount of serum protein was lower. There were no significant changes in percentage of albumin or alpha or beta globulin; and therefore these values were not thought to be of consequence in the etiology of congenital hydrocephalus.

**RÉSUMÉ**

Recherches sur la pathogénie de l'hydrocéphalie chez le rat nouveau-né déficient

On constate que l'étiologie des hydrocéphales est la même dans l'hydrocéphalie provoquée par les déficiences en vitamine B$_{12}$, avec ou sans addition d'acide X-méthylfolique. Les observations tendent à confirmer l'idée que l'hydrocéphalie est causée par la sténose de l'aqueduc cérébral associée à l'aplasie de l'organe sous-commissural et la malformation d'autres structures nerveuses. Les recherches biochimiques ne montrent aucune différence entre les types anormaux et les témoins, en ce qui concerne le volume du caillot des globules du sang, la densité spécifique de l'urine, ou la quantité de potasse du sérum. Le sodium sérique et urinaire est plus important chez les animaux déficients, mais la quantité de protéine sérique est plus petite. Il n'y a pas de
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changements significatifs dans le pourcentage de l'albumine ou des globulines α ou β — et, en conséquence, ces valeurs ne paraissent pas jouer de rôle dans l'étiologie de l'hydrocéphalie congénitale.

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