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Studies in Neural Tube Defects II. Pathologic Findings in a Prospectively Collected Series of Anencephalics

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This report presents the pathologic anatomy of a prospectively collected series of 36 anencephalic infants. This series provides an opportunity to investigate the epidemiology of organ system pathology in anencephaly (AN) as well as other facets of its natural history. AN infants had a mean gestational age 2.5 weeks younger than normal controls, though birthweight was normal for gestational age. Nearly ⅓ of the liveborn infants with AN died within 15 minutes, ⅔ within 3 hours; 3 AN infants survived to 48 hours. Details and discussions of the pathologic findings and their physiologic significance are presented. Regarding those AN infants who received detailed neurologic examinations, correlations are made between the brain pathology and neurologic function prior to death.

Key words: anencephaly, pathology, neurologic function, prospective sample, natural history

INTRODUCTION

In a previous communication [Myrianthopoulos and Melnick, 1987] regarding this prospective study of neural tube defects (NTDs) we presented an analysis of the epidemiologic characteristics of NTDs that occurred in singletons born in the Collaborative Perinatal Project. We also examined a number of pregnancy variables for possible associations with increased risks for NTDs and discussed their etiologic significance. The incidences, sex ratios, and racial distributions were similar to those reported in other study populations. Of over 50 pregnancy variables examined, 8 were found to have a statistically significant association with NTDSs: diabetes mellitus, organic heart disease, chronic lung disease, diuretics, antihistamines, sulfonam-

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ides, polyhydramnios, and abruptio placentae. In addition there was a significant risk for NTDs if the mother had a short prior pregnancy interval, especially if the prior pregnancy ended in spontaneous abortion.

This communication presents the pathologic anatomy of those NTDs in this prospective study that were diagnosed as anencephaly (AN). The wide variety of central nervous system and other organ system anomalies associated with anencephaly has long been of great fascination to embryologists, teratologists, and clinicians. The extensive literature on this subject has been thoroughly reviewed by Lemire et al [1978], who also presented data on a randomly ascertained series of 56 infants with AN. The present report adds to the existing data base a series that is unusual in that it was prospectively ascertained and thus lends itself to an analysis of the epidemiology of organ system pathology associated with AN as well as other facets of its natural history.

MATERIALS AND METHODS

The data for this study come from the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke (NCPP). The NCPP is a large-scale prospective multi-disciplinary study of factors and conditions that affect parents before and during pregnancy, and their relation to the outcome of pregnancy. Its design and population characteristics are given in the first paper of this series [Myrianthopoulos and Melnick, 1987].

The autopsies and pathological examinations were performed by NCPP pathologists at the participating institutions. The brains, when available, were given special examination. About 1,100 brains were in a condition that allowed some degree of analysis. Of these, 425 were selected by Professor P.I. Yakovlev in Boston for whole brain sectioning. The remaining brains were prepared by paraffin processing by Dr. L. Lipkin in Bethesda. Details about this material and its analysis are found in Gilles et al [1983].

The descriptive pathology and other analyses in this report will be confined to singletons born with AN. The NCPP population includes 36 newborn infants with AN, of which 32 underwent careful autopsy. Necropsies were performed and organ weights recorded according to extensive, standardized protocols established for the NCPP (manuals available on request). Normal body and organ weight values for comparison with AN specimens were taken from Brenner et al [1976], and Gruenwald and Minh [1960]. Gestational age was calculated in weeks from the first day of the last menstrual period.

Comparisons of continuous variables were accomplished by t or t' tests, depending on whether the total variances for the samples were homogeneous or heterogeneous. Analysis of discrete variables was performed by contingency χ^2, with the Yates correction for small sample sizes when appropriate.

RESULTS AND DISCUSSION

Of the 36 AN infants, 19 (53%) were liveborn and 17 (47%) were stillborn. Regarding gestational age, 19 (53%) were premature ($<$ 38 weeks), 6 (17%) were postmature ($>$ 42 weeks), and 11 (30%) were of normal gestation (38–42 weeks). The mean gestational age of all AN infants was 36.77 $\pm$ 5.03 weeks. As compared to a randomly selected control group [Myrianthopoulos and Melnick, 1987], which

had a mean gestational age of 39.21 ± 2.24 weeks, the AN group was significantly younger ($t' = -2.89$, $P < 0.01$) by approximately 2.5 weeks. There were no significant differences ($t = 1.08$, $P > 0.20$) in gestational age between liveborn infants with AN (37.63 ± 4.11) and stillborns with AN (35.82 ± 5.87).

About 72% of the AN pregnancies were associated with polyhydramnios, there being no difference between liveborn (74%) and stillborn (71%). Curiously, although 79% of the premature AN births and 82% of the mature AN births exhibited polyhydramnios, only 33% of the postmature AN births did so; this was of borderline statistical significance ($\chi^2_2 = 4.91$, $0.10 > P > 0.05$). It has been suggested that postmaturity and absence of polyhydramnios are related to a decrease or absence of adrenal activity [Anderson et al, 1971]. Since there is a well-known relationship between anencephaly and atrophy of the fetal adrenal cortex (see below), we chose to investigate the relationship between gestational age and adrenal weight as expressed in standard deviational units above or below the mean for age [Gruenwald and Mihn, 1960]. The product-moment correlation ($r = 0.36$) was not significantly different from 0 ($P > 0.05$) and, thus, no such relationship could serve as explanation.

Birthweight calculations are problematic in newborns with AN. Approximately 11–13% of the total newborn birthweight is brain-weight [Jordaan, 1976]. With substantial amounts of brain tissue missing in infants with AN, it becomes almost impossible to make meaningful comparisons with standard tables of birthweight-by-gestational age. In an attempt to investigate whether AN infants have abnormally low birthweight, we "corrected" the birthweight by adding the mean-for-gestational age brain-weight to each AN birthweight. We then plotted each case against the respective means and standard deviations for the AN infant's gestational age. Thus, the new birthweight value for each AN is expressed in standard deviational units, positive or negative, regardless of its numerical value. In this way, we found that only 2 infants with AN were more than 2 standard deviations (SD) below the mean-for-gestational age, both being liveborn. Overall, the mean deviation was less than −0.5 SD units. Although we have some reservations about our assumption of adding mean brain-weight, it is still somewhat surprising that newborns with AN, live or stillborn, do not seem to have significantly low birthweights. This would support the observations of others that, in contrast to juvenile growth, fetal growth is independent of growth hormone [Liggins, 1976], for more than 50% of our cases had no identifiable pituitary. Rather, insulin is the most important "growth hormone" of the fetus [Liggins, 1976; Thorsson and Hintz, 1977], and this is normal in AN [Cavallo et al, 1981].

Of considerable interest is the survival time of those liveborn anencephalic infants. Table I is the lifetable constructed for the 19 liveborn AN infants in this study. These data show that nearly ⅔ of the liveborn AN infants died within 3 hours (nearly ⅓ in the first 15 minutes). By 12 hours only 3 AN infants (16%) remained alive, and did so until shortly beyond 48 hours. These survival data differ substantially from those reported for a retrospective cohort by Baird and Sadovnick [1984]. They found that of their total cohort of 181 liveborn AN infants, nearly 43% survived longer than 24 hours, as compared to our population's 16%. The difference between the 2 studies is statistically significant ($\chi^2 = 5.13$, $P < 0.025$), and may be explained by either biased ascertainment of the retrospective cohort of Baird and Sadovnick or better perinatal care.

What now follows is a presentation and discussion of the pathologic anatomy found in this study population of AN newborns, and, where possible, the physiology.

TABLE I. Lifetable for Infants With Anencephaly*

Age interval (hours)	Ox	Dx	Qx	Px
0	19	12	0.63	1.00
3	7	1	0.14	0.34
6	6	0	0.00	0.32
9	6	3	0.50	0.32
12	3	0	0.00	0.16
24	3	0	0.00	0.16
48	3	3	1.00	0.16
72	—	—	—	0.00

*Abbreviations: Ox, No. alive at start of interval; Dx, No. of deaths during the interval; Qx = Ox/Dx; Px, cumulative survival at start of interval.

Central Nervous System and Neurocranium

Most investigators believe that anencephaly results from failure of the neural folds in the cranial end of the neural plate to fuse and form the forebrain and associated bony cranial vault [Giroud, 1977]. From the published literature, the variability of the central nervous system (CNS) pathology appears to be related to the extent of the original lesion and gestational age [Lemire et al, 1978].

Table II provides some details regarding the type of neurocranial defect and brain pathology. With respect to the skull anomaly, information was available for 31 of 36 AN infants: 11 (35%) had meroacrania, 4 (13%) had holoacrania, and 16 (52%) had holoacrania with rachischisis of the spine. Thus, nearly ⅔ had a malformation of the foramen magnum and more than ½ showed evidence of failed neural tube closure extending caudally to varying degrees.

Of the 16 cases where the "brain" was weighed, only one (no. 1–66) had a normal weight for gestational age. All others were well below 3 standard deviations from the mean. Of the 36 AN infants, 19 had detailed pathologic examinations. In 13 of these (68%) no recognizable anatomic structures (cerebrum, cerebellum, etc.) were identified. What tissue was present was sometimes a disorganized mass of non-specific cell types, or the so-called "area cerebrovasculosa," a mass of thin-walled vascular channels distended with blood [Giroud, 1977]. Two of these cases with no identifiable brain structures (2–50 and 3–71) had histologically identifiable structures that resembled choroid plexus. Chaurasia [1977] considers this as evidence that the most anterior portions of the neural tube may indeed have closed, particularly if the tissue mass is in the region of the forebrain. This seems doubtful to us, for there is no evidence that neural tube closure is a prerequisite for the differentiation of choroid plexus from the mesenchymal vascular pia mater. In 5 of 19 (26%) examined brains cerebra, cerebella, and brainstems were identifiable, albeit most often dysplastic. Three of these were associated with meroacrania, one with holoacrania, and one with holoacrania and rachischisis. Four of these were in newborns of 40 or more weeks gestation, one was 22 weeks gestation. One AN of 40 weeks gestation had no cerebrum or cerebellum, but did have an intact brainstem. From the data in Table II there is no indication that the brain pathology is consistently related to either the degree of acrania or the gestational age, a finding at variance with the literature [Lemire et al, 1978]. This may be owing to chance and small sample size or to the

fact that the sample was prospectively collected and thus eliminates ascertainment bias.

Neurologic and Neuropathologic Correlations

There is relatively little information available to allow correlation of neurologic activity and the existing neuropathology. In our sample, 4 AN infants lived long enough for neurologic evaluation and also had detailed pathologic examinations of the brain. These cases pose challenging questions to both the neuroscientist and the clinician, though answers may be inconclusive.

Case 1 (no. 5–05) was a white male of 43 weeks gestation with meroacrania. No brain tissue was found above the level of the intact foramen magnum. The infant had no facial muscle movement or sucking reflex. There were intermittent and uncoordinated writhing movements of the trunk and limbs and a weak cry. The child had alternate periods of hypertonia and hypotonia with occasional and slight flexion of both legs and the right arm on stimulation. Although the fingers were held in flexion, a slight palmar grasp could be elicited. Moro reflexes, plantar flexion, bilateral ankle jerks could be elicited to a slight degree. Unaided respiration was poor, with one breathing movement every 3 minutes. The infant expired 11 1/2 hours after birth.

Case 2 (no. 1–55) was a Puerto Rican female of 43 weeks gestation with meroacrania. The cerebral hemispheres were dysplastic, highly disorganized, and exhibited none of the usual anatomic structures. The cerebellum was hypoplastic, but otherwise normal; the brainstem was intact. This newborn showed normal tone throughout. She exhibited no cry, no movement of limbs, no Moro reflex, but a continuous mild tremor. The infant died after 2 days of life.

Case 3 (no 2–55) was a Puerto Rican male of 44 weeks gestation with holacrania. The 12 g brain was a disorganized tissue mass in which parts of the cerebrum and cerebellum could be identified. The infant exhibited complete atonia with no reflexes elicitable, including the suck reflex. Respiration was shallow and very irregular. The infant expired 11 hours after birth.

Case 4 (no. 5–66) was a white male of 41 weeks gestation with meroacrania. The 11 g brain consisted of an amorphous, disorganized mass of red to yellow tissue; however, there were clear demarkations of cerebrum, cerebellum and brainstem. The infant had minimal spontaneous movement of the facial muscles and there was a weak cry. In general there was decreased motor activity. There was upper extremity hypotonia and minimal hip flexion. The pupils showed no response to light and there was intermittent seizure activity. The infant died 49 hours after birth.

It has been noted that infants whose brains degenerate to the level of the caudal rhombencephalon have only primitive functions; respiration, deglutition and defence reactions persist, but the threshold of stimulation is reduced and responses are immature [Monnier and Willi, 1947]. The cases presented above seem to support these prior findings. Although these AN infants were not necessarily brain-dead, and may even have had an intact brainstem, they were capable of little more than the most elemental and primitive reflexes.

Malformations in Other Organ Systems

Congenital malformations in organ systems other than the CNS are quite common in AN [Lemire et al, 1978]. Tables III-IX list the type and frequency of

malformations in our prospectively collected NCPP sample. All of these anomalies occur at frequencies far in excess of those found in the overall NCPP study population [Myrianthopoulos and Chung, 1974]. Some of these malformations may be thought of as secondary to the acrania, cranial base anomalies, and CNS pathology, but many are seemingly embryologically remote from the primary dysmorphogenesis.

With regard to the orofacial anomalies (Table III), unusual geometric relationships secondary to the open neural tube and/or abnormalities in neural crest migration, which normally occurs after tube closure, may explain the abnormal neurocranial

TABLE II. Pathology of the Neurocranium and Brain*

Case No.	Type	Sex	Race	Gestational age (weeks)	Brain pathology
1-05	H+R	F	W	32	Pituitary present; not otherwise examined
2-05	H+R	F	W	36	Composed of disorganized neuronal mass with multiple calcifications and many large, dilated vascular channels
3-05	M	F	W	39	Not examined
4-05	U	F	W	40	Not examined
5-05	M	M	W	43	Foramen magnum intact, no brain tissue found above this level; 25 g
6-05	U	M	W	43	59 g; not otherwise examined
7-05	M	M	W	22	Intact brain covered by transparent membrane
8-05	H+R	F	W	39	Not examined
9-05	H+R	F	W	37	40 g; membranous sac containing gray/white disorganized tissue and hemorrhagic areas
10-05	H+R	M	W	40	14 g; generally a flat, lozenge-shaped mass with 4 demarcations: 2 dysplastic cerebral hemispheres, a hemorrhagic mass covered by a serous membrane and into which normal-looking cranial nerves disappear, a dysplastic caudal mass (cerebellum); a conical mass protrudes from an enlarged sella
11-05	H	F	W	40	4 g; a very small mass of tissue above the medulla oblongata, no cerebellum, midbrain or cerebral cortex present
12-05	H+R	F	W	43	23 g; not otherwise examined
13-05	H+R	F	W	35	Not examined
14-05	H	F	W	36	5 g; no normal brain tissue found
15-05	H	F	W	34	18 g; an amorphous round mass of tissue which is highly vascularized and covered by a thin membrane; no cerebellum, falx or tentorium seen; sella absent and pituitary sits free at the base of the brain mass
1-10	M	M	W	41	Severely autolyzed; not examined
2-10	M	F	W	44	No neuronal tissue, entirely replaced by a vascularized mass
1-37	U	M	B	37	Not examined
1-45	H+R	F	B	38	Severely autolyzed; not examined
1-50	U	M	W	33	Not examined
2-50	H+R	F	W	35	4 g; consists of highly vascularized, hemorrhagic tissue with narrow rims of glial tissue resembling brain but without

continued

development and subsequent viscerocranial anomalies. The anomalous position of the bones of the facial skeleton results from their articulation with the cranial base, which is usually severely malformed in AN [Marin-Padilla, 1978]. Thus it is not surprising to find a high frequency of exophthalmos, defects of the bony nasal bridge and septum, and facial clefting. Similarly, if neural crest migration is abnormal, it is also not unexpected to see so many ear anomalies.

The heart in this group of AN infants is smaller than in normal infants. About 16% had heart weights greater than 2 SDs below the mean for gestational age; about

TABLE II. Pathology of the Neurocranium and Brain* (continued)

Case No.	Type	Sex	Race	Gestational age (weeks)	Brain pathology
					ganglion cells; numerous spaces lined by columnar or cuboidal cells resembling ependyma or choroid plexus
1-55	M	F	O	43	Cerebral hemispheres are dysplastic, highly disorganized and show no identifiable anatomic structures; cerebellum hypoplastic, but otherwise normal; brainstem intact
2-55	H	M	O	44	12 g; disorganized mass in which parts of the cerebrum and cerebellum can be identified
3-55	H+R	F	W	40	34 g; not otherwise examined
1-60	H+R	M	W	26	Not examined
2-60	H+R	F	W	32	Not examined
3-60	H+R	F	B	35	Not examined
1-66	M	M	B	39	391 g; not otherwise examined
2-66	M	F	B	32	Brain mass 1 × 1 × 0.6 cm of highly dysplastic tissue; spinal cord is a filamentous, hypoplastic structure 0.2–0.3 cm in diameter
3-66	H+R	F	O	40	60 g; consists of an irregular, vascularized mass 7 × 4 × 2.5 cm with no definitive anatomy
4-66	U	M	B	34	Not examined
5-66	M	M	W	41	11 g; consists of an amorphous, disorganized mass of red to yellow tissue; however, there are clear distinctions between cerebrum, cerebellum, and brainstem
1-71	M	F	W	33	No brain tissue identified
2-71	H+R	F	W	30	20 g; disorganized tissue mass with no definitive anatomic structures
3-71	M	F	B	33	A very small mass of tissue at the cranial base composed of stratified squamous epithelium, irregular vascular channels, and glial cells; some irregular papillary structures resembling choroid plexi could be identified
4-71	H+R	F	W	35	40 g; macerated, bilobed structure with some identifiable brain parenchyma

*Abbreviations used for type: M, meroacrania (does not involve foramen magnum); H, holoacrania (defect extends through foramen magnum); H+R, holoacrania with spinal rachischisis; U, type unknown. For sex: M, male; F, female. For race: W, White; B, Black; O, Puerto Rican.

TABLE III. Orofacial Anomalies in Anencephaly

Malformation	No. affected	Frequency (N=32)
Eyes		
Exophthalmos	14	0.44
Corneal clouding	3	0.09
Ears		
Low-set	8	0.25
Posteriorly rotated	1	0.03
Protruding	6	0.19
Dysplastic pinna	7	0.22
Nose		
Flattened nasal bridge	4	0.13
Nasal septum absent	1	0.03
Mouth		
Cleft lip and/or palate	2	0.06
Macroglossia	1	0.03
Ankyloglossia	1	0.03

TABLE IV. Skeletal and Thoracic Cage Anomalies in Anencephaly

Malformation	No. affected	Frequency (N=32)
Thoracic cage		
Absent ribs	1	0.03
Abnormal rib morphology	1	0.03
Eventration of diaphragm	1	0.03
Skeletal		
Short neck	8	0.25
Kyphosis	2	0.06
Absent hand	1	0.03
Ulnar deviation, hands	1	0.03
Arms abnormally long	1	0.03
Talipes equinovarus	5	0.16
Talipes calcaneovalgus	1	0.03

TABLE V. Cardiovascular Anomalies in Anencephaly

Malformation	No. affected	Frequency (N=32)
Abnormally patent foramen ovale	9	0.28
Hypoplastic heart	5	0.16
Hypoplastic left ventricle	1	0.03
Coarctation of aorta	1	0.03
Fibrosis of endocardium	1	0.03
Single umbilical artery	5	0.16
Persistent truncus arteriosus	1	0.03
VSD	1	0.03

TABLE VI. Respiratory System Anomalies in Anencephaly

Malformation	No. affected	Frequency ($N=32$)
Lung hypoplasia	11	0.34
Incomplete lobation	2	0.06
Single lobe, right lung	1	0.03
Bilobation, right lung	3	0.09
Extra lobe, left lung	1	0.03

TABLE VII. Gastrointestinal System Anomalies in Anencephaly

Malformation	No. affected	Frequency ($N=32$)
Dilated distal esophagus	1	0.03
Ectopic gastric mucosa in esophaguss	1	0.03
Omphalocele	5	0.16
Malrotation of intestines	3	0.09
Ectopic pancreatic tissue in pylorus	1	0.03
Ectopic appendix	1	0.03

TABLE VIII. Genitourinary System Anomalies in Anencephaly

Malformation	No. affected	Frequency ($N=32$)
Hypoplastic kidneys	3	0.09
Dysplastic kidneys	2	0.06
Polycystic kidneys	1	0.03
Hydronephrosis	5	0.16
Hydroureter	1	0.03
Atretic ureter	1	0.03
Hypoplastic renal pelvis	1	0.03
Ovarian dysgenesis	1	0.03
Ovarian hypoplasia	1	0.03
Undescended testes	4	0.13
Micropenis	2	0.06
Hyperplastic labia	1	0.03

TABLE IX. Endocrine System Anomalies in Anencephaly

Malformation	No. affected	Frequency ($N=32$)
Pituitary not found	17	0.53
Pituitary hypoplastic/ dysplastic	3	0.09
Pituitary anterior lobe only	5	0.16
Hypoplastic adrenals	30	0.94
Thyroid dysplasias	3	0.09
Pancreatic islet hyperplasia	5	0.16

45% had heart weights one or more SDs below the mean. Overall, the mean decrease in standard deviational units was −0.97, as compared to normal infants. The most common cardiovascular anomalies (Table V) were abnormally patent foramen ovale (28%), hypoplastic heart (16%) and single umbilial artery (16%). Although the frequency of cardiovascular anomalies is greater than in the general population, there is no consistent pattern of anomalies other than a generalized hypoplasia which is sometimes quite exaggerated.

Lung hypoplasia is common in AN newborns, and this was a frequent finding (34%) in our AN group (Table VI). For the entire group the mean decrease in combined lung weight as expressed in standard deviational units was −1.32, as compared to normal newborns. Nearly 60% had lung weights greater than one standard deviation below the mean for gestational age, nearly 30% weights greater than 2 SDs below the mean. The frequency of lung hypoplasia may be related to the CNS anomalies. Wigglesworth et al [1977], using fetal rabbits at 22–26 days gestation, demonstrated that the CNS plays a vital role in fetal lung growth and maturation, probably by maintenance of fetal respiratory movements. Damage to respiratory centers in the brain would obviously compromise this movement. That such centers are damaged in AN newborns is evidenced by the shallow and very irregular respiration seen in those AN infants who survive long enough to be observed (see above).

Other than lung hypoplasia, the only other lung anomalies seen in our AN group were related to lobation: incomplete lobation of either lung, uni- and bilobation of the right lung, and trilobation of the left lung (Table VI). These relatively minor malformations represent anomalous secondary branching of the primary bronchopulmonary buds sometime around the fifth week of gestation, probably owing to defective epithelial–mesenchymal interaction. The relation of this event to failed neural tube closure is an enigma. Nevertheless, decreased proximal branching of the right and left stem bronchi may also be related to the overall lung hypoplasia [Reale and Esterly, 1973].

Anomalies of the gastrointestinal systems are listed in Table VII, the most frequent being omphalocele (16%) and malrotation of the intestines (9%). Hernias were not found in our series, although these are common in other reports. The liver has been described as tending toward decreased weight at birth [Lemire et al, 1978]. For our entire AN group, the mean decrease in liver weight as expressed in standard deviational units was −0.48, as compared to normal newborns. This is not considered significant. Only 29% of the AN infants had liver weights greater than one SD below the mean for gestational age, and none greater than 2 SDs below.

Genitourinary system anomalies in our AN group were quite varied (Table VIII), the most common being hypoplastic/dysplastic kidneys (16%), and hydronephrosis/hydroureter (19%). Hypoplasias of the male genitalia have frequently been described [Lemire et al, 1978] and 2 (6%) of our AN group exhibited micropenis. As with other reported studies [Naeye and Blanc, 1971] the combined kidney weights in our AN newborns were more likely than not to be below the mean for gestational age. However, the mean decrease as expressed in standard deviational units was −0.66, a decrease of small significance. Only 26% of the AN infants had combined kidney weights greater than one standard deviation below the mean for gestational age, and only 2 infants had weights greater than 2 SDs below. Of special interest is the high frequency of renal anomalies (25%): hypoplastic, dysplastic, and polycystic kidneys, hypoplastic renal pelvis, and atretic ureter. All of these may be attributed to

defective epithelial-mesenchymal interaction between the ureteric bud and the metanephrogenic mass at various stages of renal differentiation. As with the lung maldevelopment, the relationship of this compromise of a basic embryologic process to failed neural tube closure is unclear.

Anomalies of the reticuloendothelial system in our AN group involved the thymus and spleen. About 70% demonstrated thymic weights above the mean for gestational age, 42% greater than one SD, 15% greater than 2 SDs, and 8% greater than SDs. The overall mean increase in standard deviational units was +0.92. Owing to the considerable accumulation of lymphocytes, the thymic enlargement is usually in the anteroposterior dimension with an unusually wide and prominent cortex [Giroud, 1977]. About 13% of the AN infants had accessory spleens. These represent supernumerary mesenchymal condensations between the layers of the dorsal mesogastrium. The mesenchymal cells then differentiate and form the splenic capsule and connective tissue framework and parenchyma [Moore, 1982]. Other observed anomalies of the spleen include marked lobulations in one AN infant and extramedullary erythropoiesis in another.

Perhaps the most interesting anomalies associated with AN are those of the endocrine system, for they have provided considerable information regarding fetal endocrinology. The presence or absence of the pituitary in AN is still unclear from the literature. Some report it as always present [Giroud, 1977; Grasso et al, 1980], others contend that it is almost always present but usually only represented by the anterior lobe [Lemire et al, 1978], and still others report the pituitary absent [Cavallo et al, 1981]. In our AN group, after careful anatomic dissection, the pituitary was shown in 16% of cases, and in nearly 10% of cases both lobes were present but with hypoplasia and dysplasia. It is apparent then that there is rather wide variation in the status of the pituitary, from agenesis to abnormality.

Development of the neurohypophysis (posterior lobe) occurs in parallel with the embryogenesis of the adenohypophysis (anterior lobe) and the maturation of the hypothalamus [Challis and Thorburn, 1976]. Since the hypothalamus is destroyed in AN, adrenocorticotrophic hormone (ACTH) is not secreted, even in the presence of an intact anterior lobe [Tuchmann-Duplessis et al, 1974]. The consequences for fetal adrenal development will be discussed below. Suffice it to note here, that in addition to the absence of needed hypothalamic neurosecretory stimulation of pituitary ACTH release, hypothalamic factors also appear necessary for the normal development of somatotrophic and corticotrophic pituitary cells [Begeot et al, 1977]. Although ACTH is important to normal fetal development, the human fetus appears to have the capacity to grow at a near normal rate in the total absence of growth hormone [Liggins, 1976]. Indeed, the AN fetus appears normal for birthweight. By contrast, hypothalamic factors do not appear necessary for thyrotrophic and lactotrophic pituitary cell development [Leroyer-Alizon et al, 1980; Begeot et al, 1984]. Other than a few thyroid dysplasias (Table IX), the thyroids of our AN sample were normal. Other studies have found that AN pituitary thyroid-secreting hormone (TSH)-secreting cells and the thyroid gland develop normally in the absence of the hypothalamus and are able to function if adequately stimulated [Grasso et al, 1980; Leroyer-Alizon et al, 1980; Cavallo et al, 1981]. Interestingly, extrahypothalamic brain thyroid-releasing hormone (TRH) known to be independent of the hypothalamus and synthesized in situ [Jackson and Reichlin, 1977], has been demonstrated in a cell line from the sera cerebrovasculosa of an AN fetus [Ishikawa et al, 1976].

One of the principal features of AN is adrenal hypoplasia. In our sample, 94% (Table IX) were classified as having hypoplastic adrenals, the mean decrease in standard deviational units of the combined adrenal weights being −1.86. This hypoplasia is primarily owing to a reduced fetal cortex volume. In normal fetuses of 15–21 weeks gestation, there is a relative increase in the volume of the fetal cortex, whereas in AN fetuses of similar age the fetal cortex shows a highly significant ($P < 0.0001$) degree of involution [Gray and Abramovich, 1980]. It is known that the period of fetal cortex growth in normals and involution in AN coincides with the development of the fetal pituitary-adrenal axis. This suggests that the fetal pituitary gland in AN fetuses, although capable of maintaining a near-normal definitive cortex, fails to release a trophic factor for the fetal cortex [Gray and Abramovich, 1980]. Evidence suggests that the trophic factor is ACTH [Challis and Thornburn, 1976], which in AN is not released because of absent hypothalamic neurosecretory stimulation [Cavallo et al, 1981]. This fetal cortex pathology is associated with abnormal fetal steroidogenesis, including decreased levels of pregnanetriol, 17α-hydroxypregnanolone, tetrahydro-11-deoxycortisol and tetrahydrocorticosterone [Cawood et al, 1976]. Finally, several studies have been cited in the monograph by Lemire et al [1978] which purport that the degree of fetal cortex hypoplasia is dependent on the age of the AN fetus at birth. As noted above, when we investigated the relationship between gestational age and adrenal weight, the product-moment correlation ($r = 0.36$) was not significantly different from 0 ($P > 0.05$). Thus, our study does not support prior conclusions in this regard.

Finally, it was of great interest to us to try and gain some new insight into the relationship between the severity of the neural tube defect and the malformation incidence of other organs or organ systems. This would be of value in understanding how anomalous neural tube development might influence embryogenesis in general and might also help to define heretofore unrecognized developmental fields. Given the small sample size ($N=32$), we were obliged to investigate this relationship by defining our severity groups as cranioschisis (AN) and craniorachichisis (AN + spina bifida), and to look at these 2 groups relative to organ system development rather than individual organ malformations. Multiway χ^2 tests of independence revealed that the frequency of malformation for the various organ systems (Tables III–VIII) is independent of the severity, cranioschisis vs. craniorachischisis ($\chi^2_5 = 0.14$, $P > 0.99$).

These results may be explained in at least 2 ways. First, the sample may be too small to statistically elucidate a dependent relationship between severity of the neural tube malformation and specific organ system defects. This would be particularly so if the relationship is subtle, even if biologically important. Second, actually there may be no relationship. Our simple, clinical classification of severity may be masking important embryologic phenomena. For example, Marin-Padilla [1978] has postulated that the same mesodermal insufficiency which is associated with various types of axial dysraphic disorders could affect embryos at a more advanced stage of embryonic development when the neural folds are already completely closed. Thus, the array of other organ system malformations may be quite similar in AN infants with or without rachischisis, at least for those structures dependent upon a critical density of mesoderm (and perhaps neural crest as well).

CONCLUSION

The prospective sample of AN presented here provides a reasonable epidemiologic approximation of the frequency of associated anomalies and allows for comment on their clinical relation to anencephaly, as well as their physiologic effects. Enigmas persist, however, regarding the relationship of failed neural tube closure to malformations in organ systems widely disparate in developmental time and location, good examples being lung and renal anomalies.

It has sometimes been purported that a resolution of the etiologic heterogeneity of AN awaits a more definitive understanding of the developmental sequences or fields associated with the wide phenotypic variation seen in AN infants. This is not likely to be the case, in our view, for it presupposes that there is a one-to-one correspondence between a particular pathogenetic pathway and a particular etiologic trigger. Marin-Padilla [1978] has proposed, with good experimental evidence, that impaired formation, elevation, and approximation of the neural folds is likely due to a primary disturbance of the axial chordomesodermal system, resulting in a deficiency of the necessary underlying, non-segmented mesoderm. This critical pathogenetic event, he points out, has been seen in experiments with several teratogens (eg, vitamin A, dimethylsulfoxide, and trypan blue). This is but one illustration; should we delineate pathogenesis from affected developmental fields in AN infants, it will in no way assure a clearer understanding of the etiologic heterogeneity of AN. Rather, we are more apt to have better luck in understanding AN going at it the other way around. Resolving the etiologic heterogeneity of AN will likely provide insight into the pathogenetic relationship of AN with other organ system defects. At least, this approach is more readily testable by animal experimentation.

REFERENCES

Anderson ABM, Davies LK, Campbell H, Turnbull AC (1971): Foetal adrenal weight and the cause of premature delivery in human pregnancy. J Obstet Gynaecol Br Commonw 78:481–488.

Baird PA, Sadovnick AD (1984): Survival in infants with anencephaly. Clin Pediatr 23:268–271.

Begeot M, Dubois MP, Dubois PM (1977): Growth hormone and ACTH in the pituitary of normal and anencephalic human fetuses: Immunocytochemical evidence for hypothalamic influences during development. Neuroendocrinology 24:208–220.

Begeot M, Dubois MP, Dubois PM (1984): Evolution of lactotropes in normal and anencephalic human fetuses. J Clin Endocrinol Metab 58:726–730.

Brenner WE, Edelman DA, Hendricks CH (1976): A standard of fetal growth for the United States of America. Am J Obstet Gynecol 126:555–564.

Cavallo L, Altomare M, Palmieri P, Licci D, Carnimeo F, Mastro F (1981): Edocrine function in four anencephalic infants. Horm Res 15:159–166.

Cawood ML, Heys RF, Oakey RE (1976): Corticosteroid production by the human foetus: Evidence from analysis of urine from women pregnant with a normal or an enencephalic foetus. J Endocrinol 70:177–126.

Challis JRG, Thorburn GD (1976): The fetal pituitary–adrenal axis and its functional interactions with the neurohypophysis. In Beard RW, Nathanielsz PW (eds): "Fetal Physiology and Medicine." Philadelphia: WB Saunders, pp 232–253.

Chaurasia BD (1977): Forebrain in human anencephaly. Anat Anz 142:471–478.

Gilles FH, Leviton A, Dooling EC (1983): "The Developing Human Brain. Growth and Epidemiologic Neuropathology." Boston: John Wright-PSG.

Giroud A (1977): Anencephaly. In Vinken PJ, Bruyn GW, Myrianthopoulos NC (eds): "Handbook of Clinical Neurology, Vol 30, Congenital Malformations of the Brain and Skull Part I." Amsterdam: Elsevier, pp 173–208.

Grasso S, Filetti S, Mazzone D, Pezzino V, Vigo R, Vigneri R (1980): Thyroid–pituitary function in eight anencephalic infants. Acta Endocrinol 93:396–401.

Gray ES, Abramovich DR (1980): Morphologic features of the anencephalic adrenal gland in early pregnancy. Am J Obstet Gynecol 137:491–495.

Gruenwald P, Minh HN (1960): Evaluation of body and organ weights in perinatal pathology. I. Normal standards derived from autopsies. Am J Clin Path 34:247–253.

Ishikawa H, Nagayama T, Kato C, Niizuma K (1976): Establishment of a TSH-releasing-hormone-secreting cell line from the area cerebrovasculosa of an anencephalic fetus. Am J Anat 145:143–148.

Jackson IMD, Reichlin S (1977): Brain thyrotrophin-releasing hormone is independent of the hypothalamus. Nature 267:853–854.

Jordaan HVF (1976): Newborn brain: body weight ratios. Am J Phys Anthropol 44:279–284.

Lemire RJ, Beckwith JB, Warkany J (1978): "Anencephaly." New York: Raven Press.

Leroyer-Alizon E, David L, Dubois PM (1980): Evidence for calcitonin in the thyroid gland of normal and anencephalic human fetuses: Immunocytological localization, radioimmunoassay, and gel filtration of thyroid extracts. J Clin Endocrinol Metab 50:316–321.

Liggins, GC (1976): The drive to fetal growth. In Beard RW, Nathanielsz PW (eds): "Fetal Physiology and Medicine." Philadelphia: WB Saunders, pp 254–270.

Marin-Padilla M (1978): Clinical and experimental rachischisis. In Vinken PJ, Bruyn GW, Myrianthopoulos NC (eds): "Handbook of Clinical Neurology, Vol 32, Congenital Malformations of the Spine and Spinal Cord." Amsterdam: Elsevier, pp 159–191.

Monnier M, Willi A (1947): Die integrative tätigkeit des nervensystems beim normalen säugling und beim bulbospinalen anencephalen (rautenhirnwesen). Ann Paediatr 169:289–308.

Moore KL (1982): "The Developing Human." 3rd edition. Philadelphia: WB Saunders, pp 235–239.

Myrianthopoulos NC, Chung CS (1974): "Congenital Malformations in Singeltons: Epidemiology Survey." New York: National Foundation — March of Dimes, Vol X, No 11.

Myrianthopoulos NC, Melnick M (1987): Studies in neural tube defects. I. Epidemiologic and etiologic aspects. Am J Med Genet 26:783–796.

Naeye RL, Blanc W (1971): Organ and body growth in anencephaly. Arch Pathol 91:140–147.

Reale FR, Esterly JR (1973): Pulmonary hypoplasia: A morphometric study of the lungs of infants diaphragmatic hernia, anencephaly and renal malformations. Pediatrics 51:91–96.

Thorsson AV, Hintz RL (1977): Insulin receptors in the newborn: Increase in receptor affinity and number. N Engl J Med 297:908–912.

Tuchmann-Duplessis H, Auroux M, Haegel P (1974): "Illustrated Human Embryology." Volume 3. New York: Springer-Verlag, pp 136–137.

Wigglesworth JS, Winston RML, Bartlett K (1977): Influence of the central nervous system on fetal lung development. Arch Dis Child 52:965–967.

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