Levels of α-fetoprotein in amniotic fluids of mice (curly-tail) with neural tube defects

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Summary. Mutant curly-tail mice are genetically predisposed to produce offspring with neural tube defects. Estimation of α-fetoprotein in the amniotic fluid of fetuses of these mutants has shown that the levels are raised in fetuses with exencephaly and open spina bifida. This suggests that these mice are a valid model for studies of the aetiology and genesis of neural tube defects in man.

The mutant mouse curly-tail (ct) has a high incidence of spina bifida and exencephaly. The mutation arose spontaneously in a GFF stock and the abnormalities were described by Gruneberg in 1954. The expression of the gene is variable. Mice with exencephaly or large open lumbosacral spina bifida die shortly after birth. Smaller sacral lesions may heal in the first few days of life, and the animals survive with no apparent dysfunction. However, they have a tightly coiled tail. Other mice have no overt spina bifida at birth but have a kinked or a spirally twisted tail. Gruneberg's studies show that these mice have a transitory caudal spina bifida which heals during fetal life. According to Gruneberg, however the abnormality presents itself, the primary defect is in the central nervous system, which fails to close, and the effect on the skeleton is secondary.

These mice seem to be an animal model for human neural tube defects, and they have potential for the study of the genesis, aetiology, and many other aspects of the condition. In order to see if these mice even begin to mimic human neural tube defects, we have examined the amniotic fluids of the mouse embryos, to see if open lesions are associated with raised levels of α-fetoprotein (AFP). In humans this is so, and it forms the basis of a successful diagnostic test for the antenatal detection of neural tube defects (Brock and Sutcliffe, 1972; Brock and Scrimgeour, 1972; Seller et al, 1973; Seller, 1974).

Materials and method

The estimation of AFP in curly-tail mouse amniotic fluids was carried out using the single radial diffusion method (Mancini et al, 1965). Immune serum against mouse AFP was obtained by injecting rabbits with a partially purified preparation of mouse AFP. The specificity of the immune serum was confirmed using a rabbit anti-mouse AFP kindly donated by Dr S. Sell (Department of Pathology, San Diego, La Jolla, USA). The levels of AFP in the curly-tail mouse amniotic fluids are expressed as a percentage of the AFP present in a pool of amniotic fluid obtained from a normal strain of mice, the A Strong, which do not have a special propensity to neural tube defects. This is the standard AFP.

The amniotic fluid from each individual fetus from the mutant mice were separately collected at 12, 14, 15, 16, 17, 18, and 19 days of gestation. The position of each fetus in the uterus was recorded, its condition noted, and assigned to one of three groups. Those termed 'normal' were those without an open tube defect. They were mice with a curly or kinky tail or no visible tail defect, any lesion of the neural tube having already completely healed. The second group were those with an open spina bifida. The third group were fetuses with exencephaly or exencephaly and spina bifida. A detailed analysis of the various types of matings and the incidence of the malformations will be reported elsewhere.

The samples were collected and coded by one person and the AFP assayed by another person.

Results

The mean levels of AFP in the amniotic fluids of curly-tail mice classified as 'normal', that is without an open spina bifida, were found to increase with

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gestational age (Fig. 1 and 2), the highest levels occurring at term.

There were 20 fetuses with exencephaly, and when their amniotic fluid AFP levels were compared with those of 'normal' mice of the same gestational age, 11 were found to be greater than twice the standard deviation of the mean (Fig. 1). The presence of exencephaly could be detected by the raised AFP levels at all stages of fetal life studied: abnormal values occurred in 12-day as well as 19-day-old exencephalic fetuses.

Of 15 fetuses with open spina bifida, the amniotic fluid AFP levels of 5 of them exceeded twice the standard deviation of the mean (Fig. 2). Though the number of affected fetuses is small, the association of abnormal levels of amniotic fluid AFP and spina bifida seemed to be more frequent in 18- to 19-day-old fetuses. None of the younger spina bifida fetuses showed high levels of AFP in the amniotic fluid.

Discussion

These observations indicate that the curly-tail mouse is like the human in that open neural tube defects are associated with raised levels of AFP in the amniotic fluid. There are minor differences, but these can probably be accounted for by the relative immaturity of the mouse at birth compared with humans. In 'normal' curly-tail mice without an open spina bifida, the amniotic fluid levels of AFP increase with gestational age and are maximal at birth, whereas in humans, the amniotic fluid AFP levels of normal fetuses reach a peak at around the 15th week of gestation and decline thereafter (Gitlin and Boesman, 1966). This differing situation in the mouse presumably reflects the synthesis of AFP which increases right up to the time of delivery. High levels of AFP are still present in the sera of newborn mice and rats and their values decline only a few weeks after delivery (Abelev, 1971; Wepsic and Sell, 1974). By contrast, in man, AFP synthesis in the fetus decreases markedly in the third trimester and serum levels at birth are around one-thirtieth of those prevailing around the 15th week of gestation when the serum levels are at their peak (Gitlin, 1975).

Our findings indicate that the curly-tail mice are genetically predisposed to produce neural tube defects which resemble the human condition, and work is in progress to attempt to elucidate the role that inherited and environmental factors play in the aetiology of the malformation, and to study the embryogenesis of the lesions.

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REFERENCES
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Announcement

Dr Heinz Karger Prize 1976

The prize which is awarded every year in memory of Heinz Karger, the well-known Basle publisher, for an outstanding scientific work, has in 1976 been conferred in equal parts on D. J. H. Brock (Scotland) for his paper 'Protein Measurements in the Early Prenatal Diagnosis of Spina Bifida' and P. R. Wyatt/D. M. Cox (Canada) for their paper 'The Utilization of Electron Microscopy in the Prenatal Diagnosis of Genetic Disease'.

The Dr Heinz Karger Memorial Foundation invites the submission of papers on the following subjects:

1977: An original research paper on 'Molecular Biology of Metabolic Diseases'.
1978: An original research paper on 'Cytological and Histochemical Approach to the Diagnosis of Tumours'.

Conditions

Manuscripts shall not exceed 20 typewritten pages, including illustrations, tables, and bibliography. Manuscripts marked 'Competition' must reach the publishers, S. Karger AG, Arnold-Böcklin-Strasse 25, CH-4011 Basle (Switzerland), not later than 28 February 1977 and 1978. The manuscript must be typewritten on one side only, double-spaced, and is to be submitted in quadruplicate, and in accordance with the instructions contained in the 'Rules for the Preparation of Manuscripts'. This leaflet can be obtained free of charge from the publishers if the request is marked 'Competition'.

Language: English, German, or French.

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