Ectopic Brain Tissue in Laboratory Rats

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Introduction

In man ectopic brain tissue in a paracranial location is not a rare occurrence (*Suneson & Kalimo* 1979, *Call & Baylis* 1980, *Okulski et al.* 1981, *Ofodile et al.* 1982, *Wakai et al.* 1983, *Zook et al.* 1984, *Seibert et al.* 1984) while brain tissue at other locations is infrequently reported (*Gonzales-Crussi et al.* 1980) and mostly associated with anencephaly (*Chen et al.* 1982, *Woznial & Rozynek* 1984). Ectopic brain tissue is on the other hand only occasionally reported in animals (*Varnum & Fox* 1981).

At necropsy of 11 days old Sprague-Dawley rats, in connection with a pharmacological experiment, a glandlike structure was found medial and caudal of the scapula on the right chest wall in several animals (medial of latissimus dorsi and teres major and lateral of serratus ventralis). In sixteen cases the glandlike structure was prepared for histopathological diagnosis at the Unit for Laboratory Animals at the National Veterinary Institute, Uppsala. All the glandlike structures appeared to consist of brain tissue with most parts of the brain represented. It was decided to check the circumstances and to try to follow up the occurrence of this ectopic brain tissue.

Materials and methods

Animals: The initial discovery was made in October 1984 (week 43), in connection with a pharmacological experiment, on Sprague-Dawley rats from Møllegaard Ltd. This investigation, initiated by the initial finding of ectopic brain tissue, covers in total 256 litters comprising 2141 offspring (Fig. 1). The presence of ectopic brain tissue was recorded during a period ranging from week no. 43, 1984 until week no. 41, 1985 (Fig. 1 & 2).

During 1984 only rats from Møllegaard Ltd. were used but in January 1985 female and in February 1985 male Sprague-Dawley rats from ALAB (Laboratorietjänst AB) were introduced.

Special consideration was focused on the circumstances and the occurrence of this anomaly during weeks 7–25, 1985 (Fig. 1 & 2, Table III).

The investigation also included a cross mating study of 76 litters, using the four possible combinations resulting in a production of 432 offspring (Tables I & II). This material, however, was a part of the total material scored during the weeks 7–25 (Fig. 1 & 2, Table III).

During week 18 and 34, 1985, litters from 15 bipara females were investigated. These females were again covered with the previously used males and had all in their first litter 3, or more, offspring with ectopic brain tissue.

Ten of the same females were mated a third time with the same males and the litters were investigated at week 41, (Fig. 1).

When mated, one male was kept with two females during five days. After that the male was removed. The two females were kept together one additional week, whereafter, on the 12th day, they were put into separate cages.

With the exception of weeks 18, 34 and 41, 1985 only primipara females were used. The males, however, were used several times during approximately 5 months.

When mated the first time, the weight of the females was about 200 g and of the males

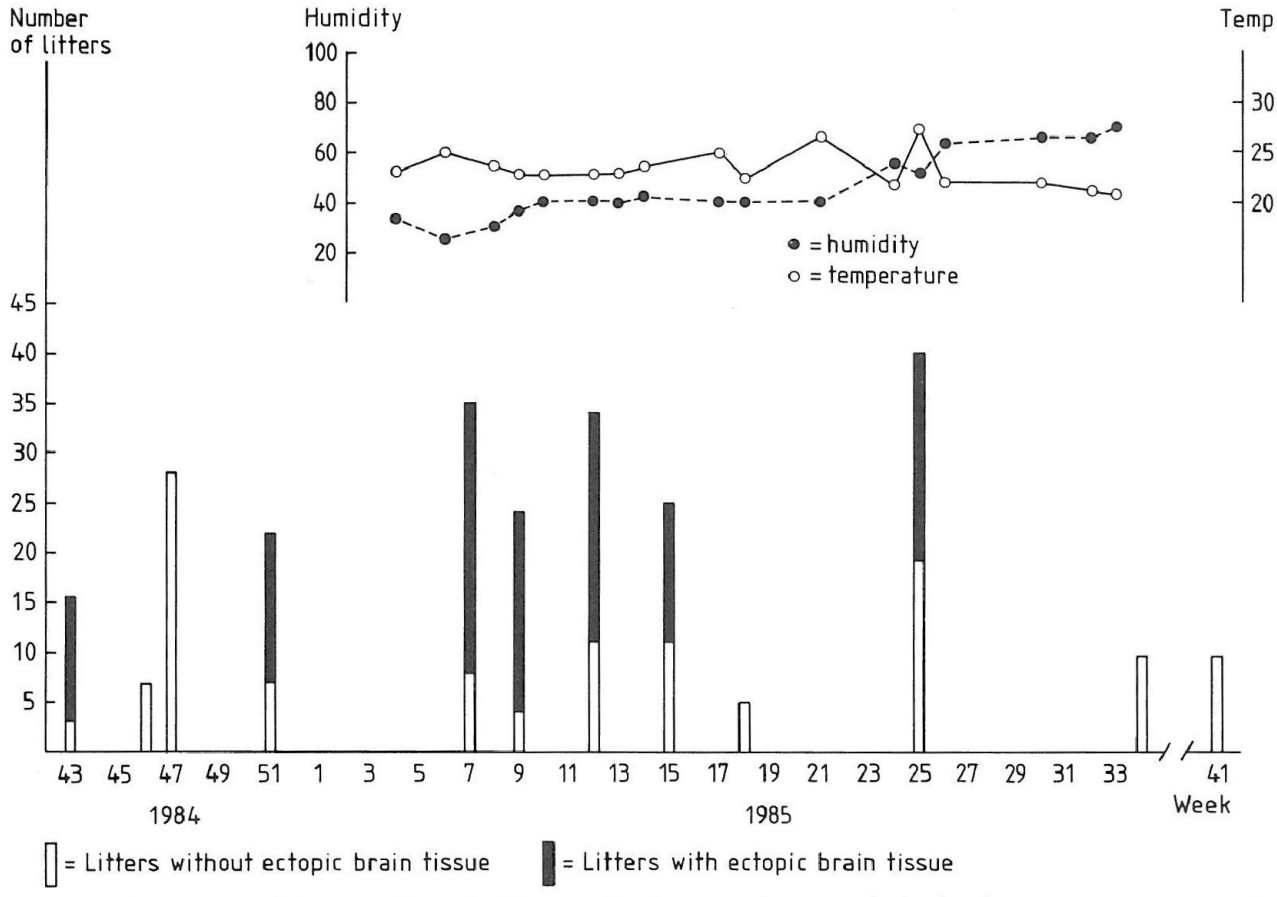

Fig. 1. Distribution of litters with and without offspring carrying ectopic brain tissue. Measurements of humidity and temperature.

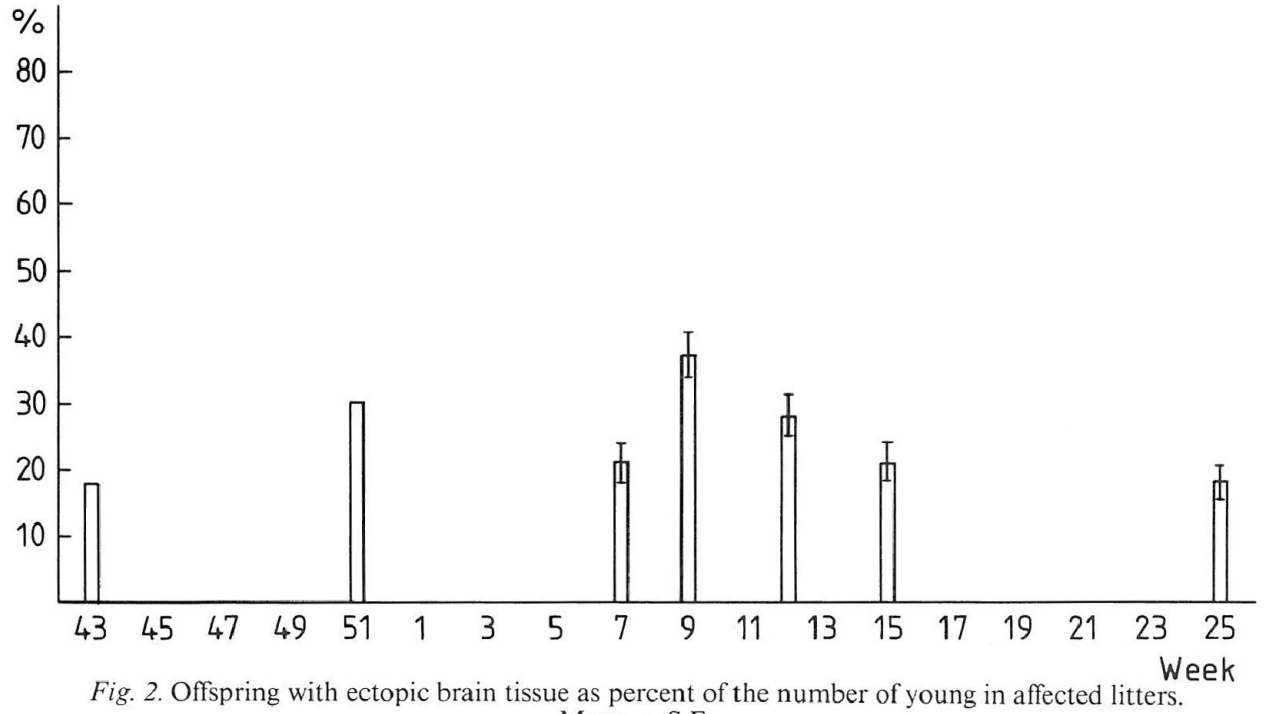

Fig. 2. Offspring with ectopic brain tissue as percent of the number of young in affected litters. Mean ± S.E.

Table I. Distribution in the breeding programme of litters with cases of ectopic brain tissue.

Litters	Combinations* ♂ × ♀ A A	♂ × ♀ A M	♂ × ♀ M A	♂ × ♀ M M
with offspring having ectopic brain tissue	7 (78 %)	30 (64 %)	1 (50 %)	13 (72 %)
with all offspring normal	2 (22 %)	17 (36 %)	1 (50 %)	5 (28 %)
total number of litters	9	47	2	18

* A = ALAB rats
M = Møllegaard rats

Table II. Distribution of offspring with ectopic brain tissue in litters with this malformation.

Offspring	Combinations* ♂ × ♀ A A	♂ × ♀ A M	♂ × ♀ M A	♂ × ♀ M M
with ectopic brain tissue	16 (26 %)	70 (28 %)	1 (17 %)	26 (22 %)
normal	46 (74 %)	177 (72 %)	5 (83 %)	91 (78 %)
total number of young	62	247	6	117

250 g. The litters were recorded at birth and on the fourth day the number of offspring in each litter was adjusted to a maximum of 10.

The pharmacological experiment did not involve any treatment of the mothers, but administration of different pharmacological substances to the offspring four hours before euthanasia on the 11th day p.p.

Bedding material: Originally Hanflock H 3/4 was used. Torrax (ALAB) was introduced in December 1984. The bedding was changed twice a week. In connection with the parturition the bedding was changed 4

Table III. Frequency of affected young.

Scoring period Weeks No. (1985)	No. of affected young	No. of young in affected litters	Total number of young	Frequency of affected young of the total No. of offspring
7	47	223	342	13.7 %
9	67	180	205	32.7
12	51	185	275	18.5
15	28	134	239	11.7
25	36	200	388	9.3

days before. Four days after parturition the female and her litter were moved to a clean cage.
Fodder: Pellets (Altromin, Standard-Diät, Brogaarden) and tapwater (in bottles) ad lib.
Environment: Humidity and temperature were measured during spring 1985 (Table 1).
Euthanasia method: Due to experimental demands (of the pharmacological experiment) all animals were killed by means of cervical dislocation. Animals were euthanasied on day 11.
Pathology: Initially, selected tissues from 16 animals were sent fixed in 4 % formaldehyde for histo-pathological examination. Later on 20 animals were subjected to more detailed necropsy. Visceral organs, brain and ectopic brain tissue were fixed in 4 % formaldehyde. The material was embedded in paraffin, cut in 5 μ thick sections and stained with haematoxylin eosine and according to Holmes. The relative weight of the brain was determined in 50 animals without ectopic brain tissue and in 49 animals with ectopic brain tissue.

Results

Glandular like structures were found only on the right chest wall medial and caudal of the scapula (Plate 1). The histological investigation revealed brain tissue of mixed origin, mainly cerebrum and cerebellum but also ependymal cells and choroid plexus, appearing in a partly disorganized pattern (Plate 2). Meninges were not found (Plate 3). No other anomalies were found and all other tissues examined were macro- and microscopically normal. The distribution of litters in which ectopic brain tissue was found and litters with normal offspring is seen in Fig. 1. Ectopic brain tissue was found in 296 offspring from 133 litters. The frequency of offspring with ectopic brain tissue varied between 10–70 percent in affected litters. Average percentage is shown in Fig. 2. The results of the mixed breeding between ALAB and Møllegaard rats are presented in Tables I and II. The figures presented in Table II give no evidence for a rejection of the 0-hypothesis, stating that there is no statistical difference between the frequences of individuals with ectopic brain tissue from the four mating combinations (chi-square = 1.823; $d_f = 3$). Thus, in the statistical analysis, made on the total offspring produced during the weeks 7 to 25, no consideration has been taken to the origin of their parents.
The environmental checks of relative humidity and temperature are presented in Fig. 1. During the period between week no. 7 to week no. 25 (1985) frequences of affected young among the offspring were scored as presented in Table III. By means of chi-square calculations on the values in that table and also from Fig. 2, it is fully evident that there is a significant positive peak in the frequency of affected young scored during week no. 9 with a strongly decreasing slope during the following weeks. The relative weight of the brains (given as percent) was in the 50 normal animals: 4.36 ± 0.06, and in the 49 animals with ectopic brain tissue: 3.82 ± 0.09, given as Mean and Standard Error. Thus the normal animals had significantly heavier brains than the affected ones ($p < 0.001$).
The attempt to reproduce litters with ectopic brain tissue failed completely, though animals were used in combinations that earlier had produced more than 30 percent anomalous offspring. Not one single out of 260 offspring revealed any anomaly. In addition, after week 25, 1985, no case of this anomaly has been found.

Discussion

It is apparent that the glandular structures found were ectopic brain tissue and most probably emanating from a displacement during early embryogenesis of neuroectodermal cells with potential for differentiation. It is known that one of the most vulnerable incidents in embryology is the separation of the neuroectoderm from the epithelial ectoderm. Failure of invagination of the primitive medullary plate to proceed in an or-

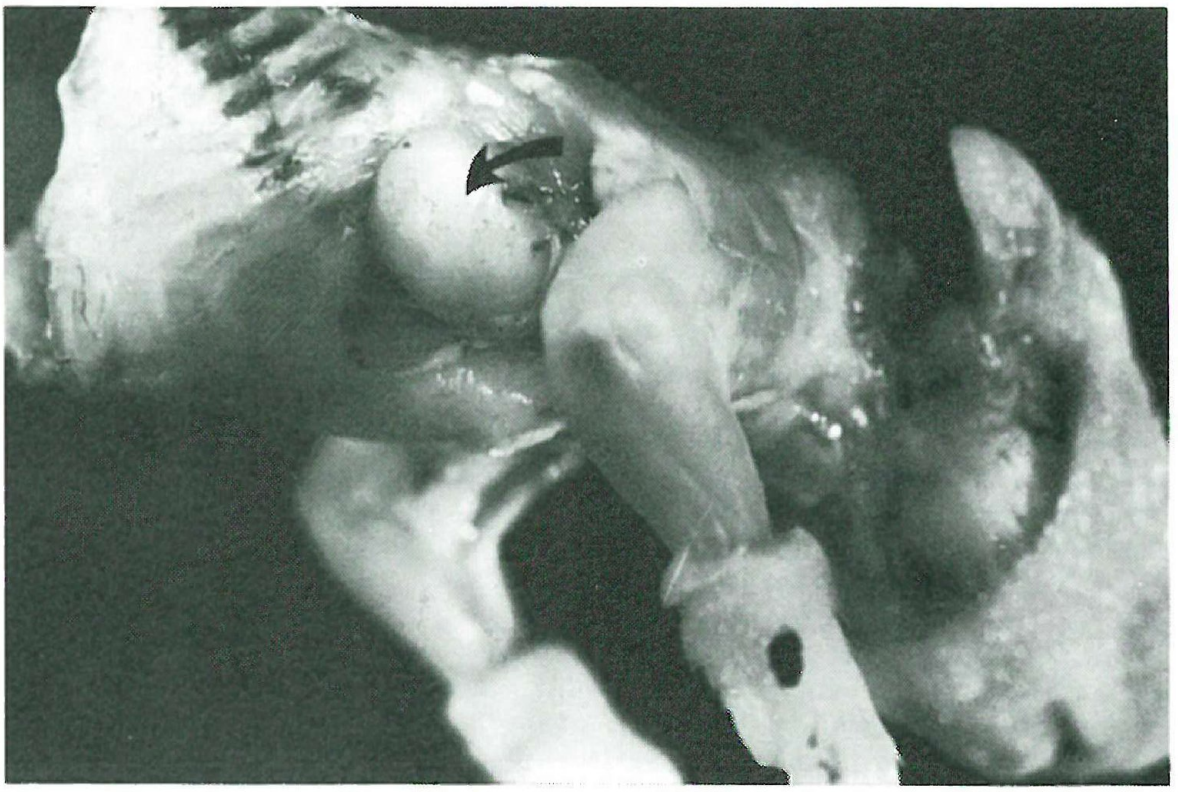

Plate 1. Glandlike structure on the right chestwall.

Plate 2. Margin of glandlike structure. To the right brain tissue (B) of a disorganized pattern. At F, brown fat at M, striated skeletal muscle. HE × 110.

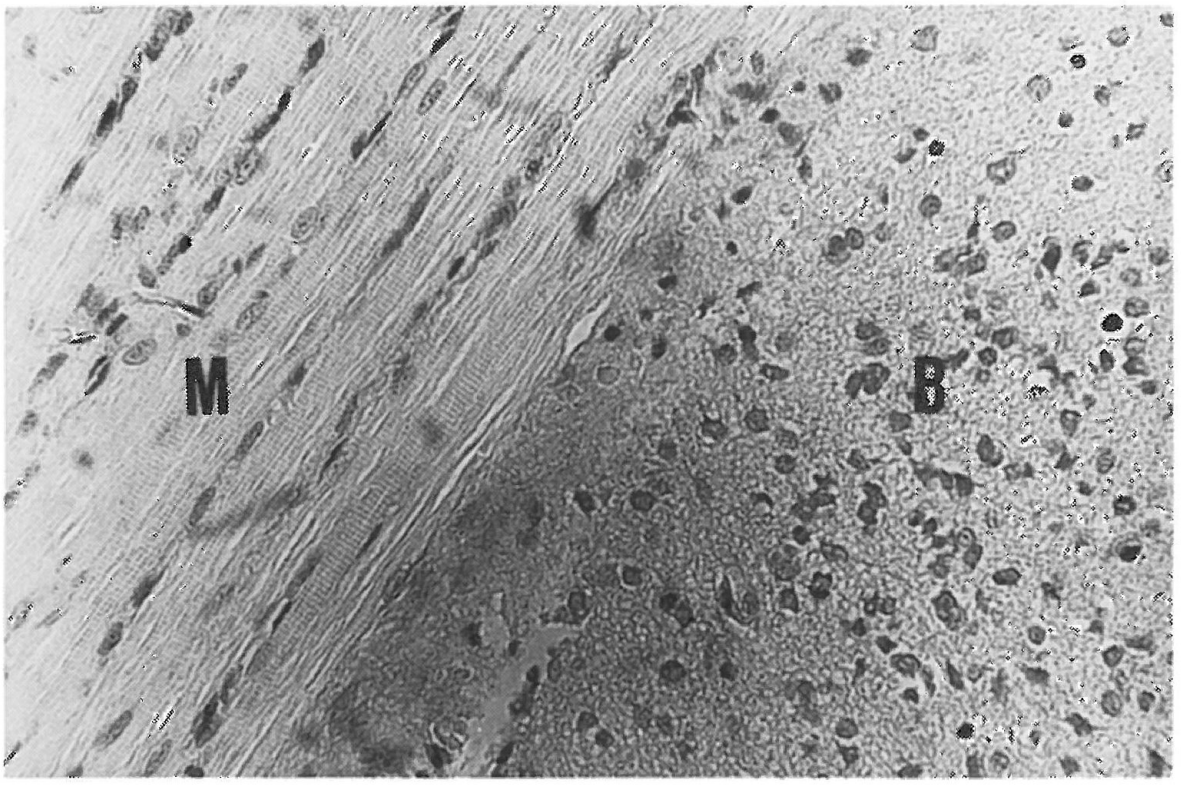

Plate 3. Margin of glandlike structure. Note close contact between brain tissue (B) and striated muscle (M) and absence of meninges. HE × 240.

derly and complete fashion may result in a wide variety of defects involving the skin and central neural axis as well as the surrounding structures of mesodermal origin (*Matson & Ingraham* 1951). Primary dysraphias, that is, failure of closure of some part of the embryonic neural tube, however, is reported to be extremely rare in rats (*Wilson* 1978) and the authors have not found any earlier reports on ectopic brain tissue in rats.

One may postulate that a defect causing displacement of neuroectodermal cells, capable of differentiating into glial elements and choroid plexus, ought to have occurred at a very early embryonic stage. Specifically, it is during organization of the neural tube, perhaps before the 25–30 somite stage, that a pinching off, of both neural crest cap and medullary epithelium would be particularly apt to occur. It should be noted that only the medullary epithelium can act as a precursor of choroid plexus structures, and that once neuroectodermal epithelial cells are engaged in neuroblastic or glioblastic differentiation, there is no possibility that they may be transformed into choroid plexus (*Lee et al.* 1976). Hence the finding of a lower relative weight of the brains in animals carrying ectopic brain tissue indicates a development error in which neuroectodermal cells, capable of differentiation have been "pinched off" and displaced early in embryonic development. It has been stated that migrating neural crest cells do not seem to display predetermined patterns of differentiation, but that this in large measures the result of local inductive influences (*Weston* 1970). Hence the disorganized pattern of the ectopic tissues could depend on such inductive influences.

A genetic background appears less probable considering the irregular frequency with which the ectopic brain tissue has appeared.

The variations in time and within litters (10–70 percent), as well as the use of males and females from different sources, the restricted extent of the anomaly and also the failure to reproduce the ectopic brain tissue using the same parents point in the same direction.

The only report on genetically produced ectopic brain tissue in rodents, found by the authors, is on a mutation on chromosome 4 of the mouse. (*Varnum & Fox* 1981). This mutation, when homozygous, produced prenatal blebs (usually on the head), open eyelids and folded retinas at birth. Extra toes or ectopic brains were occasionally observed.

Thus exogenous parametres, such as various infectious agents, chemicals, toxins and environmental factors have to be considered.

Virus infections are reported to induce malformations of the developing nervous system. These malformations are usually symetric, non inflammatory and lacking neuropathologic features of a previous viral infection (*Kilham & Margolis* 1966, *Johnson* 1971). As the animals, at the actual premises, were neither health monitored nor screened for viral infections the microbiological status remains unknown. The animals did, however, appear clinically healthy. The parents did not show any abnormal sexual behaviour. No reduction in litter size, was found. No other anomalies were found in the offspring but for the unilaterally carried ectopic brain tissue. A virus infection, early in fetal development, as a possible cause or as a part of a multifactorial genesis can, however, not be ruled out.

Chemicals of different kinds are unavoidable parameters in the milieu of laboratory animals. Despite this there is little known how these compounds affect the laboratory animal.

The bedding material may contain substances having significant effects on animals biological responses. Thus bedding material made from pine and cedar is known to cause changes in hepatic microsomal enzymes of mice and rats (*Krafi* 1980). High pup mortality among rats, attributed the use of cedar-wood shawings as bedding, is reported (*Burkhart & Robinson* 1978).

Also soaps and detergents, used for the cleaning of cages and water bottles, may produce subtile or obvious biological effects depending on the level of exposure (*Burek & Schwetz* 1980).

Medical treatments of different kinds may also be considered. Diverse compounds e.g. several anthelmintics (*Philip & Birkhead* 1974, *Belatour et al.* 1975) are well known to have teratogenic effects.

Mycotoxins, as feed contaminants, are also capable of producing prenatal teratogen effects (*Newberne & Fox* 1980). Ochratoxin A is reported to produce a spectrum of malformations, among those exencephaly (*Arora* 1982).

The bedding material was changed during the investigation. In spite of this, litters with and without ectopic brain tissue were produced. On the other hand the cleaning and washing procedures were the same during the whole time of the investigation. It seems thus less likely that these two exogenous factors have played any role in the patogenesis of the anomaly.

The enhanced temperature and the low relative humidity encountered (Fig. 1) may have led to an increased intake of water. The drinking water available was ordinary tapwater that was not monitored neither for chemical nor microbiological contamination. Hence the role of the water and the waterconsumption remains unclear.

The fodder was not known to have any added medical compounds but none of the batches used were submitted to microbiological check and were not tested for fungal growth. Thus the possibility of a toxic substance in the fodder has to be considered one possibility in the pathogenesis of the ectopic brain tissue, either alone or as a parameter in a multifactoral genesis.

Several environmental factors are recognized to have profound effects on the biological response of animals to various experimental

conditions (*Pakes et al.* 1984). In addition severe behavioral and physiological stress during gestation such as conditioned anxiety, crowding, immobilization and temperature extremes, may permanently modify the structural or functional development of offspring in rats. Under certain conditions these stresses produce an aberrant sexual behavior in male offspring (*Ward* 1972, *Whitney & Herrenkohl* 1977) and reduced fertility and fecundity in female offspring (*Herrenkohl* 1979).

The environment for the rats in the present study was not controlled. The only environmental check performed, during a limited period, concerned temperature and humidity (Fig. 1). It is stated that room temperature between 22°–24°C is desirable and that relative humidity should not fall below 40 percent (*Kohl & Barthold* 1984). It is evident that, during parts of the time when these parameters were measured, the temperature was too high and humidity much too low. In litters, scored during week 9 a significant increase in the frequency of ectopic brain tissue cases was observed (Figs. 1 and 2). The largest discrepancies, from desirable environmental conditions, were measured at week 6 when these females were mated. After temperature and humidity were returned to normal values the anomaly was not seen any more and was not able to reproduce although animals earlier producing offspring with ectopic brain tissue were used. It is noticable, however, that ringtail was not encountered. Ringtail is a disease of infant rats attributed, among other things, to low relative humidity and fatty acid deficiency (*Njaa et al.* 1957, *Totton* 1958, *Dikshit & Siramachari* 1958, *Kohn & Barthold* 1984). It is apparent, however, that prenatal stress, produced by unfavourable climatic conditions, has to be considered one possible parameter in the patogenesis of the ectopic brain tissue. Thus there are many factors that might have played an important role in the patogenesis. Many of these as the microbiological status of the animals, food quality and most environmental factors, do remain unrevealed. Hence it seems best to summarize by stating that the patogenesis of the ectopic brain tissue is still, at large, unknown.

The demonstrated lack of knowledge concerning as well the environment as the microbiological status of the animals, food, bedding etc. underlines that health monitoring is a mandatory procedure for the identification of health problems affecting laboratory animals.

Summary

Ectopic brain tissue was detected in 11 days old rats. The anomaly was found medial and caudal of the scapula on the right chest wall appearing as a glandlike structure. Animals with ectopic brain tissue were found to have a lower relative brain weight indicating a development error in which neuroectodermal cells had been "pinched off" and displayed early in embryonic development. Several etiological possibilities are discussed, i.e. genetic background, virusinfections, chemicals, toxins and environmental factors. A correlation between a situation with the parameters of high temperature and low humidity versus an increased frequency of the anomaly seemed possible. As the microbiological status of the animals, food quality and most environmental factors were not known it was stated, however, that the patogenesis of the ectopic brain tissue is still unknown.

Sammanfattning

Ektopisk hjärnvävnad påvisades hos 11 dagar gamla råttungar. Denna anomali förelåg medialt och caudalt om bogbladet på höger sidas bröstvägg och såg ut som en körtelliknande struktur. Djur med ektopisk hjärnvävnad befanns ha en lägre relativ hjärnvikt indikerande ett utvecklingsfel varvid neuroektodermala celler hade blivit "avklippta" och felorienterade tidigt i den embryonala utvecklingen. Ett flertal etiologiska möjligheter diskuteras såsom genetisk bakgrund, virusinfektioner, kemikalier, toxiner och omgivningsfaktorer. En möjlig korrelation förelåg mellan hög temperatur och låg fuktighet i djurrummen och en ökad frekvens av ektopisk hjärnvävnad. Då emel-

lertid uppgifter om djurens mikrobiologiska status liksom fodrets kvalitet och de flesta omgivningsfaktorer ej förelåg är patogenesen för den ektopiska hjärnvävnaden fortfarande ej klarlagd.

Yhteenveto / K. Pelkonen

Työssä tutkittiin 11 päivän ikäisissä rotissa löytyvää ektooppista aivokudosta. Anomalia pikallistuu lapaluusta mediaalisesti ja kaudaalisesti rintakehän oikealle puolelle ja muistutti rauhaskudosta. Niillä eläimillä, joilta löytyi ektooppista aivokudosta, oli ruumiinpainoon suhteutettuna kevyet aivot. Löydös viittaa kehityshäiriöön, jossa neuroektodermaalisia soluja on kuroutunut irti ja kulkeutunut väärään paikkaan varhaisessa sikiönkehityksen vaiheessa. Työssä pohditaan useita etiologisia mahdollisuuksia, mm. geneettistä taustaa, virusinfektion mahdollisuutta, toksiineja ja ympäristötekijöitä. Vaikuttaa mahdolliselta, että ilmiössä voi löytyä positiivinen korrelaatio korkean lämpötilan ja matalan ilmankosteuden yhteisvaikutuksen kanssa. Loppupäätelmä on kuitenkin, että ektooppisen aivokudoksen patogeneesi jää vielä tuntemattomaksi, koska eläinten mikrobiologinen status, ruuan latu ja useimmat ympäristötekijät eivät olleet tunnettuja.

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