Inheritance and morphology
of exencephaly, a neonatal lethal recessive with partial
penetrance, in the house mouse

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SUMMARY

An inherited exencephaly in the mouse is described, using embryos at 11–17 days gestation. The cephalic portion of the neural tube remains open on the dorsal surface, the bony vault of the brain fails to develop, and the tissues of the brain are exposed; the development of these tissues, which is somewhat variable, is described. There is strong evidence that the condition is due to a single recessive gene, xn, with full viability until birth. Penetrance is twice as high in females as in males, and it varies overall according to the genetic milieu studied, from 33 % to 84 %. These features, and the absence of pleiotropic effects and of genetic evidence of associated chromosomal anomalies, make its inheritance different from that of exencephalies so far described.

1. INTRODUCTION

Exencephaly, as an inherited condition claimed to be without pleiotropic effects, was first described by Bonnevie (1936), who called it pseudencephaly. Carter (1956, 1959) believed that it was related to the myelencephalic bleb condition (*my*) which arose in the same stock. Grüneberg (1952) tolerated Bonnevie's claim: in discussing the work of Snell, Bodemann & Hollander (1934), where pseudencephalics occurred in conjunction with X-ray induced translocations, he suggested that there are several different genetic situations giving the same defect, due to disturbance of embryonic development at the same critical stage. Since Bonnevie's stock is extinct, controversy remains.

The mechanism of brain ectopia is also uncertain. Contrary to current opinion, Bonnevie believed that her defect was due to increased tension owing to abnormal curvature of the neural tube in the cervical region; but Carter (1959) held that there was a primary inability of the neural tube to close.

More recently, Brown & Harne (1973, 1974) has described a phenotype similar to Bonnevie's called 'anencephalus', detectable by failure of neural tube closure

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at the 16–18 somite stage. It occurred in a strain carrying open eyelids with cleft palate (*oel*, Gleucksohn-Waelsch, 1961*a*, *b*), and was at first thought to be gentically related to the latter. This proved to be a developmental interaction in which expression of *oel* does not occur in anencephalus. He has since established the anencephalus condition in a separate line where it is 100 % penetrant and 86 % viable to birth (Brown, 1977). A review of teratogenically induced ectopias is given by Kalter (1968).

Morphologically, Bonnevie's pseudencephaly closely resembles the subject of this paper, and the incidence in her stock was similar: she assumed it to be due to a single recessive gene, but heterozygotes gave about half the expected one-quarter affected.

Anencephaly associated with curly-tail and spina bifida, probably a single recessive, *ct*, is described by Grüneberg (1954) as due to delayed closure of the neural tube. It is similar morphologically to the present condition and also in that the incidence is higher in females than in males (Seller, 1978); it has been proposed as a model for human anencephaly (Searle, 1959).

The condition described in this paper is definitely due to a single recessive gene, is not associated with other neural tube defects, is due to failure of neural tube closure, and has morphological features differing from those of all similar conditions so far described.

2. MATERIAL

Breeding programme

During a pilot study of the mutagenicity of DDT (Wallace, Knights & Dye, 1976), exencephalous young were found at 16 days gestation during the autopsy of 170 females in the 11th generation of a DDT-treated strain of very prolific random-bred CF/1 albino mice. Twelve females carried these affected young, the overall ratio normal:affected being 8:1. In newborn litters from parents of these, several young were seen to have the tops of their heads missing; as they occurred in about the same proportion in segregating litters, it was suggested that these were suffering the same defect (then mistakenly called 'hydrancephalus') and that the condition is lethal at birth (Knights, 1972).

The stock was maintained by matings between sibs of affected young. Affected mice were detected by placing pregnant females in a cage with a grid shelf 1 in. above the bottom; this prevented the mother's access to them, but let the young fall onto soft warm material below. By frequent inspection of the pregnant females from 9 a.m. to midnight, litters were observed so recently born that deaths were due mainly to the affected condition. Affected members, however, were not all dead, but the exposed brain was highly vascular and they died within hours; it was clear that the exposed brain was damaged during parturition, and it may be that mothers with access to affected young eat the brain because of its olfactory resemblance to the placenta.

3. METHODS

Breeding programme

A stock of about 15 pairs of carriers of the defect was maintained as follows. Assuming the condition to be recessive, *xn*, known carriers of a given generation were used at first to detect carriers among their progeny; then known carriers within the progeny were used as test-mates; and finally carriers of each sex were mated at random to form the new generation. The young from the test-matings were born in grid cages and classified for sex and affected status. They were first litters of the animal being tested (or the second, when the first, by mistake, was not born in a grid cage). Animals from subsequent litters were born in our standard cages (design: Wallace, 1965) and were set aside to be tested for the new generation. No genetic status is implied by the classification as carrier and non-carrier, simply the success or failure to pass this test.

In the earlier or 'maintenance' generations, further autopsies at 16 days gestation were carried out on carrier females mated to carrier males. This stock was then expanded to seven trios (called selection generation 0). For selection generations, 1, 2, 3 and 4, pairs numbering 20, 10, 13 and 12 respectively, were mated, the members of the matings being those who had had the highest number of affected young in test-matings.

A carrier was crossed to curly-tail, *ct*, to test allelism. Another carrier from the maintenance stock (albino brown, non-agouti: *cc bb aa*) was outcrossed to a tan ragged stock (a^t, *Ra* – chromosome 2), and carrier F_1 $a^t a$ *Ra* + crossed to carrier *aa Ra* +, and also backcrossed to carrier maintenance stock. Similarly, a carrier maintenance mouse was crossed to Danforth's short-tail (*Sd* – at the end of chromosome 2 opposite to *Ra*), and *Sd* + carrier F_1 backcrossed to maintenance carriers. Finally, carrier selection generation 1 and 2 females were outcrossed to mice of phenotype hammer-toe, sombre, twirler, black, short-ear dilute, agouti (respectively *Hm* – chromosome 5, E^{so} – chromosome 8, *Tw* – chromosome 18, *B* – chromosome 4, *se* and *d* – chromosome 9, *A* – chromosome 2). Carrier F_1 hammer-toe sombre twirler mice were mated to carrier F_1 mice normal for these mutants, and also backcrossed to selection generation 2 carriers.

The young, born in standard cages, provided data from which linkage of exencephaly with all the chromosome markers above, and albinism (*c* – chromosome 7), could be detected. $F_1 \times F_1$ provided intercross data, and $F_1 \times$ maintenance or selection stock, backcross data.

Morphology

Pregnant females were sacrificed at known gestation times from 10 to 17 days (counting day plugged as day 0). The embryos were dissected from the amniotic sacs, fixed in 10 % buffered formol-saline, and examined with a dissecting microscope. The exencephalic defect was visible at 10 days gestation, but the external details were more clearly visible after application of a few drops of methylene blue dye to the exposed neural tissue. After fixation embryos were embedded in

paraffin wax, sectioned and stained with haematoxylin and eosin. Approximately half the abnormal embryos were sectioned coronally and the remainder in the sagittal plane. The smaller embryos were serially sectioned at 5 μm. Sections were taken at 500 μm intervals from the larger 17-day embryos. Fourteen abnormal embryos were examined and normal litter-mates were treated in the same way to act as controls.

4. RESULTS

Breeding programme

(a) *Inbreeding section*

The numbers of normal and affected mice (all alive) in segregating litters of the earliest maintenance generations, obtained by autopsy of females in known carrier × carrier matings, are given in Table 1. The numbers in segregating litters,

Table 1. *Numbers of normal and affected young in segregating litters of the three main CF/1 stocks*

	Normal	Affected	Total	Affected (%)
Autopsies, maintenance	185	31	206	10·19
Grid, maintenance	1436	239	1675	14·27
Grid, selection generations 0–4	1741	511	2252	22·69
Totals	3362	771	4133	18·65

2×3 heterogeneity $\chi^2 = 56 \cdot 24$ for 2 D.F., $P \ll 0 \cdot 001$.

Table 2. *Numbers of normal and affected young, by sex, in segregating litters of the maintenance generations*

Normal		Affected		Total	Females: affected (%)	Males: affected (%)
♀	♂	♀	♂			
680	756	153	86	1675	18·37	10·21

2×2 contingency $\chi^2 = 21 \cdot 97$ for 1 D.F., $P \ll 0 \cdot 001$.

born in the grid cage (alive and dead) from successful test-matings, of the later maintenance generations, represent the result of a modest amount of selection, and are shown next in this table. Lastly, the numbers in segregating litters, born in the grid cage from successful test-matings, in selection generations 0–4 inclusive, are given: these represent the strongest selection results. There is a significant heterogeneity in the normal:affected observations, the incidence of affected doubling over time; this proves that selection is effective. Since the CF/1 stock was very inbred before the random-mating immediately preceding the appearance of exencephaly (Wallace *et al.* 1976), any modifiers must be few in number. A single-locus shift in gene frequency is more plausible (see below).

A disparity between the sexes as regards affected status was seen in maintenance generations born in the grid: the breakdown of these data is given in Table 2.

The disparity is significant. Since in total the sexes are equal, it is more likely to be due to different penetrance in the sexes than to unequal viability. The female sex doubles penetrance.

A breakdown of data for young born in the grid cages, classified as alive ordead as well as for sex, are available for selection generations 2–4 inclusive. These are

Table 3. *Numbers of live and dead young in segregating and non-segregating litters of selection generations*

A. Data include affecteds

	Females		Males			Data label
	Alive	Dead	Alive	Dead	Total	
Gens. 2–4: segregating litters	491	450	588	267	1796	(a) +
Gens. 2, 3: non-segregating litters	68	49	67	35	219	(b)
Totals	559	499	655	302	2015	(c)

2×4 heterogeneity $\chi^2 = 2.13$, $P > 0.5$.

B. Data include and exclude affecteds

	All females	All males	Males (%)	
Gens. 2–4: segregating litters	941	855	47·6	(a) +
Gens. 2, 3: non-segregating litters	117	102	46·6	(b)
Gens. 2–4: as above but excluding affected	660	747	53·1	(a) −
Totals for data (a) + and (b), i.e. (c)	1058	957	47·5	

2×2 contingency χ^2 on data (a) + and (b) = 0.56, $P > 0.3$.
2×2 contingency χ^2 on data (a) − and (b) = 3.23, $P \sim 0.05$.
χ^2 testing equality of sexes for data (c) = 5.06, $P < 0.05$.

given in Table 3 for segregating litters; there are also data from some generation 2 and 3 litters, from the same source, which did not segregate. Table 3, data A, shows that the death-rate in the grid cages was the same for both sexes whether exencephaly was segregating or not; this indicates that exencephaly was the only sex-controlled cause of deaths. The disagreement in percentage males when exencephalics are excluded (second two lines, data B) confirms this. There is also a significant but small difference between males and females overall (line 4 of data B). The first two lines of data B show agreement between segregating and non-segregating litters in the size of the deficit of males, and proves that deaths before birth are unconnected with exencephaly; the agreement in incidence of affecteds at autopsy and at birth, mentioned under 'Materials' above, supports this. The general conclusion may now be firmly drawn that there is no tendency for affected individuals of either sex to die before 16 days gestation or between it and birth. A difference in incidence of affected between sexes is thus, as suspected above, entirely due to sex-limitation.

The numbers of tested animals for each of generations 0–4 which did not

produce affected young in grid cages (non-carriers) and which did produce them (carriers) are given in Table 4. This gives a new measure of the effectiveness of selection within the selection stock. It shows that the percentage of non-carriers identified in the four generations declines; this trend is not quite significant.

Table 4. *Numbers of carriers and non-carriers in selection generations 0–4*

Selection generation	(♀ and ♂ pooled)		Total	Non-carriers (%)
	Carriers	Non-carriers		
0	69	10	79	12·65
1	133	17	150	11·33
2	82	3	85	3·53
3	117	7	124	5·65
4	22	1	23	4·35

2×5 heterogeneity $\chi^2 = 7·92$ for 4 D.F., $P > 0·05$.

	Carriers		Non-carriers		
Totals	♀	♂	♀	♂	Total
	194	229	24	14	461

2×2 contingency $\chi^2 = 3·62$ for 1 D.F., $P > 0·05$.

Table 5. *Numbers of litters segregating and not segregating in selection generations 0–4, compared with expectations on incidence of affecteds*

	No. litters not segregating	No. litters segregating	Total
Observed	24	194	218
Expected	15·26	202·74	218

χ^2 test of fit $= 5·39$, $P < 0·02$.

It might be thought that the non-carriers in Table 4 were genetically able to produce affecteds, but did not do so owing to the limitation of litter size. The expected number of non-segregating litters, due to chance and not to non-carrier status of a parent, on the basis of litter size and observed incidence of affecteds, is given in Table 5. (These expectations are obtained as follows: the average litter size per female non-carrier in Table 4 was 12·79, and the average per female carrier was 12·32. The proportion of affected young out of all litters, including non-segregators, is 1/5 or 20·54 %. The chance of no affected appearing in a litter of 12, from a mating capable of producing them but not doing so, is then $(4/5)^{12}$, which is 0·07. Account is taken of litters of each size, and overall expectations for the segregating and non-segregating litters are obtained.)

Table 5 shows that the observed number of non-segregating litters significantly exceeds the expected number, and that at least nine (24–15) out of 218 litters were genuinely from a non-carrier parent. But the most striking finding in Table 5

is that as *many* as 194 or more litters out of 218 came from a carrier parent. If exencephaly is, as assumed, due to a single recessive gene, *xn*, each segregating litter proves that one parent is a carrier (the other parent is, as explained under 'Methods', proven carrier by a different mating). Putative parents for the next generation were drawn from the sibs of exencephalics: if all *xnxn* die neonatally, then only 2/3 of parents should be carriers, not about 93 %. It can only be concluded, therefore, that a large number of *xnxn* survive to become parents. The incidence of less than a quarter affected in segregating litters is thus due, wholly or in part, to impenetrance of *xnxn*; and the shift in incidence of affecteds in Table 1, and in the incidence of carriers in Table 4, are also fully explicadle on this basis, i.e. an increase in the incidence of *xnxn* relative to $+ xn$ and $+ +$. (The slight difference between the sexes regarding carrier status – bottom of Table 4 – can also now be understood. Many carriers are *xnxn*. The incidence of lethal *xnxn* is higher in females: thus the number of adult female *xnxn* carriers is reduced). This finding has been reported (Wallace & Anderson, 1976).

It should be pointed out that a polygenic hypothesis which excludes the existence of a major gene does not fit the observations. For example, if two recessives were necessary, only double heterozygotes could produce affecteds, and these would occur in 4/15 sibs of affected, which is even further from 93 %. A dominant gene or genes is also precluded (see section (*b*) below).

A full breakdown of the progeny from segregating litters in selection generations 2–4 (not given in tabular form) shows that there is a significant increase in both sexes of affected young (18–26 %), as expected from an increase in the frequency of *xn* over $+$; but there is also a shift in female progeny from the dead to live category, i.e. a change in expression. This indicates some genetic modification. As pointed out, the number of modifiers must be small due to the inbreeding; they could be due to the (relatively low) mutagencity of DDT.

(b) *Outcrossing section*

The cross of a carrier to curly-tail (*ctct*) produced no exencephalics out of 50 progeny, thus disproving allelism and dominance. The carrier selection generation females crossed to multiple-mutant stock produced 0/65 female young and 0/55 male young, confirming recessivity. The subsequent segregations of carrier parents and affected progeny are given in Table 6.

In data A, there is an excess of carrier F_1 over that expected on the assumption that the carrier outcrossed is $+ xn$, but the excess fits the assumption that this carrier is *xnxn* unexpressed. The significant heterogeneity in the output of selection stock test-mates in data A shows that there was more than one genotype of test-mate. The first gave equal numbers of affecteds and normals, and the others about 1:4. With all F_1 $+ xn$, the first test-mate must have been *xnxn* and the others $+ xn$. On this assumption penetrance of *xnxn* in the first case must have been $13/(\frac{1}{2}$ of 28), or 93 %, and in the second, about $19/(\frac{1}{4}$ of 103), or 74 %. The figures are small enough to agree with an overall penetrance of about 84 %. They do not agree with the supposition that a sizeable fraction of *xnxn* die before

16 days gestation. (Bonnevie invoked early embryonic death to explain her low incidence of affecteds, but did not report any dead embryos recognizable as affected.) In addition data A further precludes dominance. The outcrossed

Table 6. *Segregations on outcrossing to a normal stock and backcrossing to the carrier stock*

A. Results of outcrossing and test-mating F_1

Carrier albino: ref. no.	Multiple-mutant stock	F_1 ($\times$ carrier albino) proven by progeny to be				F_1 origin	Progeny of $F_1 \times$ test-mates	
		Carrier		Non-carrier				
		♀	♂	♀	♂		Aff.	Nor.
1. ♀	$\times$ ♂	2	1	–	–	Son of 1. ♀	13	15
2. ♀	$\times$ ♂	4	2	–	–	Sons and daughters of 2. ♀	16	62
3. ♀	$\times$ ♂	–	1	1	1	Son of 3. ♀	3	22
4. ♀	$\times$ ♂	1	–	–	–	Son of 4. ♀	Data lost	
	Totals	7	4	1	1	F_1 of 2. ♀, 3. ♀	19	84

χ^2 for 2 D.F. testing heterogeneity in the last column is 9·93 ($P < 0·01$).
χ^2 testing $(7+4):(1+1)$ for $1:1 = 6·23$, $P < 0·02$.
χ^2 testing $19:84$ for $1:3 = 2·36$, $P > 0·20$.

B. Carrier status of backcross progeny, and segregation from their test-matings

Carrier albino: ref. no.	$\times$	F_1 from outcross above	Backcross progeny (B_1) proven by progeny (B_2) to be				B_2 progeny	
			Carrier		Non-carrier			
			♀	♂	♀	♂	Aff.	Nor.
5. ♂	$\times$	♀	–	1	–	–	2	8
6. ♂	$\times$	♀	–	3	–	–	4	25
7. ♂	$\times$	♀	4	2	–	–	9	51
8. ♂	$\times$	♀	5	1	1	–	11	40
9. ♀	$\times$	♂	–	1	–	–	2	10
		Totals	9	8	1	1	29	134

χ^2 testing $(9+8):(1+1)$ for $2:1 = 4·45$, $P < 0·05$.
χ^2 testing $29:134$ for fit to $19:84 = 0·02$, $P > 0·9$.

carrier, if $Xn+$, could not have been expressing the Xn gene; imperfectly pene-trating dominants are partial recessives, and it is common experience that their homozygotes are more extreme in expression than the heterozygotes. In this case they would probably all die, so the outcrossed carrier could not be $XnXn$: yet only if it is homozygous can it produce more than half of F_1 carriers, as observed.

With regard to data B of Table 6, and reverting to the assumption of death

of 84 % *xnxn*, if the genotypes of the F_1 × selection generation carrier were +*xn* × +*xn* then they would give backcross progeny (B_1) carrier : non-carrier in the ratio 2 : 1. The observations 17 : 2 disagree significantly. However, if these carriers, as shown above, are +*xn* or *xnxn*, F_1 × carrier matings would be a mixture of +*xn* × *xnxn* (giving all carriers) and +*xn* × +*xn* (giving 2/3 or 3/4 carriers, depending on penetrance); the observed 17 : 2 fits this expectation. The agreement of the affected : normal status of the B_2 progeny of data B with that of the progeny of test-matings of F_1 in data A, is also in keeping with a high but not perfect penetrance of *xnxn*, and no antenatal death of *xnxn*. More complex models than the present single-gene one do not fit all the facts so well; the latter simpler model is now assumed to be apt.

The high penetrance of *xn* after outcrossing may now be compared with penetrance within the selection stock. Assuming no antenatal death, a rough estimate of the penetrance in the latter may be made as follows. The incidence of + + in the population is (24 + 14/461) from Table 4, i.e. 8 %. Since only carriers were mated, a Hardy–Weinberg distribution is not, strictly, to be expected, but it is not far out (when +*xn* and *xnxn* were randomly mated): where + + has a low frequency, it exaggerates the fraction of + + in the next generation only a little. With 8 % + + in one generation then, the Hardy–Weinberg distribution of the three genotypes in the next is

+ +	+*xn*	*xnxn*
0·08	0·41	0·51

Taking the incidence of affected as 388/1796 (selection generations 2–4) = 0·17, the maximum penetrance of *xnxn* is 0·17/0·51, or 33 %. This is very much lower than the 84 % found after outcrossing. Modification of penetrance by genetic milieu is thus proved.

Table 7. *Number of young bred from intercross and backcross data for each of the markers being tested for linkage*

	A,a^t,a	*Ra*	*Sd*	*Hm*	E^{50}	*Tw*	*B,b*	*se,d*	*c*
Intercross	55	253	—	—	—	—	56	70	200
Backcross	60	100	145	360	326	264	120	—	480

The numbers of young classified for intercross and backcross data for each of the markers is given in Table 7. Owing to the intercross segregation of exencephaly in all these matings, a linkage would be detected by disturbance of the normal ratios of the markers, due to the proximity of a marker or its allele to this 84 % lethal condition. There was no disturbance in any of the segregations, even in the female progeny where the highest proportion of *xnxn* would die. Close linkage is therefore precluded and *xn* cannot yet be assigned to a particular chromosome.

Morphology

Exencephaly in the *xn* mouse is a condition resulting from abnormal development of the cephalic portion of the neural tube. The neural tube is open on the dorsal surface on day 10, the bony vault of the cranium fails to develop and the tissues of the brain are exposed (Plates I and II). The neural tube is opened out, bringing structures normally in the floor and lateral walls of the brain to the surface. The exposed neuroectoderm fuses laterally and ventrally with the head ectoderm.

In the *xn* stock there is a variation in the exencephalic defect dependent on the extent of opening of the neural tube. In all embryos the entire diencephalon (thalamus) and mesencephalon (midbrain) is open. In the 10-day embryo, a stage when the neural tube has normally closed, a mid-line slit-like aperture is visible in the roof of the mesencephalon. In slightly older embryos the defect is more obvious and the diencephalic and mesencephalic roof is folded laterally and downwards forming a cap on the surface of the brain. The cavity of the third ventricle is severely narrowed in the coronal plane and in some embryos partially obliterated by fusion of the thalamus across the midline. There is variable extension of the opening anteriorly to involve parts of the telencephalon (cerebral hemispheres) and posteriorly to involve the mesencephalon (pons and cerebellum) and the myelencephalon (medulla). There is no constant relationship between the extent of the anterior and posterior defect.

(a) Anterior variation

In the most severely affected embryos the telencephalic vesicles have formed normally as outpouchings from the third ventricle, but are displaced ventrally and posteriorly and almost entirely covered by the cap of out-turned thalamus. The third ventricle opens to the surface through a wide central anterior aperture (Text-fig. 1) and the orifices of the lateral ventricles (foramina of Munro) are visible externally in the lateral margins of the aperture. The lateral choroid plexus has formed within the lateral ventricles, but in some animals a small part of the plexus, on one or both sides, protrudes through the lateral ventricles and is continued downwards for a short distance over the surface of the brain to fuse with the head ectoderm. In all embryos the telencephalic vesicles are collapsed and in a proportion there is a fold in the floor of the lateral ventricles, which appears to be due to lateral compression and downward displacement of the apex of the vesicle. The corpus striatum is partially fused in the midline and bulges upwards and forwards into the anterior opening of the third ventricle (Plate III). The limit of the anterior opening is formed by the anterior wall of the telencephalon – the lamina terminalis.

In the least severe anterior defect (Text-fig. 2) the surface is again formed by the out-turned thalamus covering the telencephalic vesicles, but the lateral ventricles open normally into the compressed third ventricle. The lateral ventricles are not visible externally and the choroid plexus is not exposed. The external
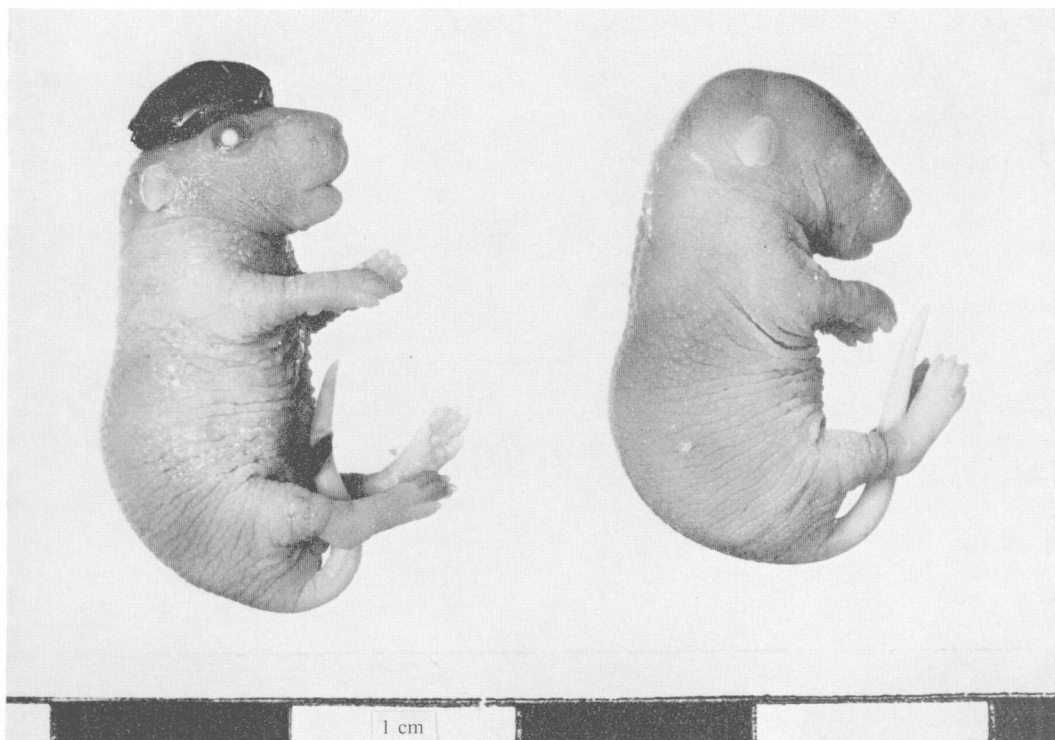
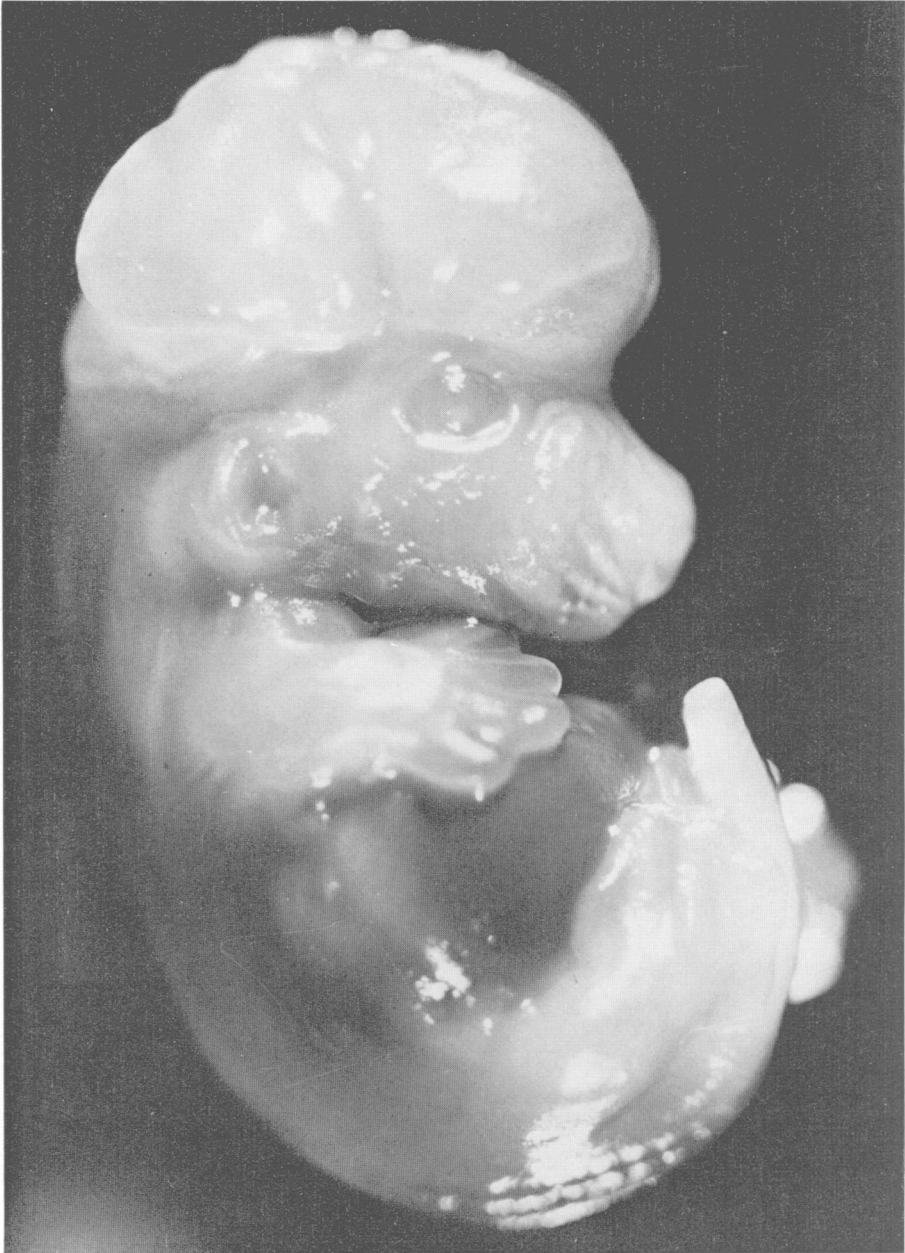Plate I. Exencephalic mouse and normal litter-mate after birth.

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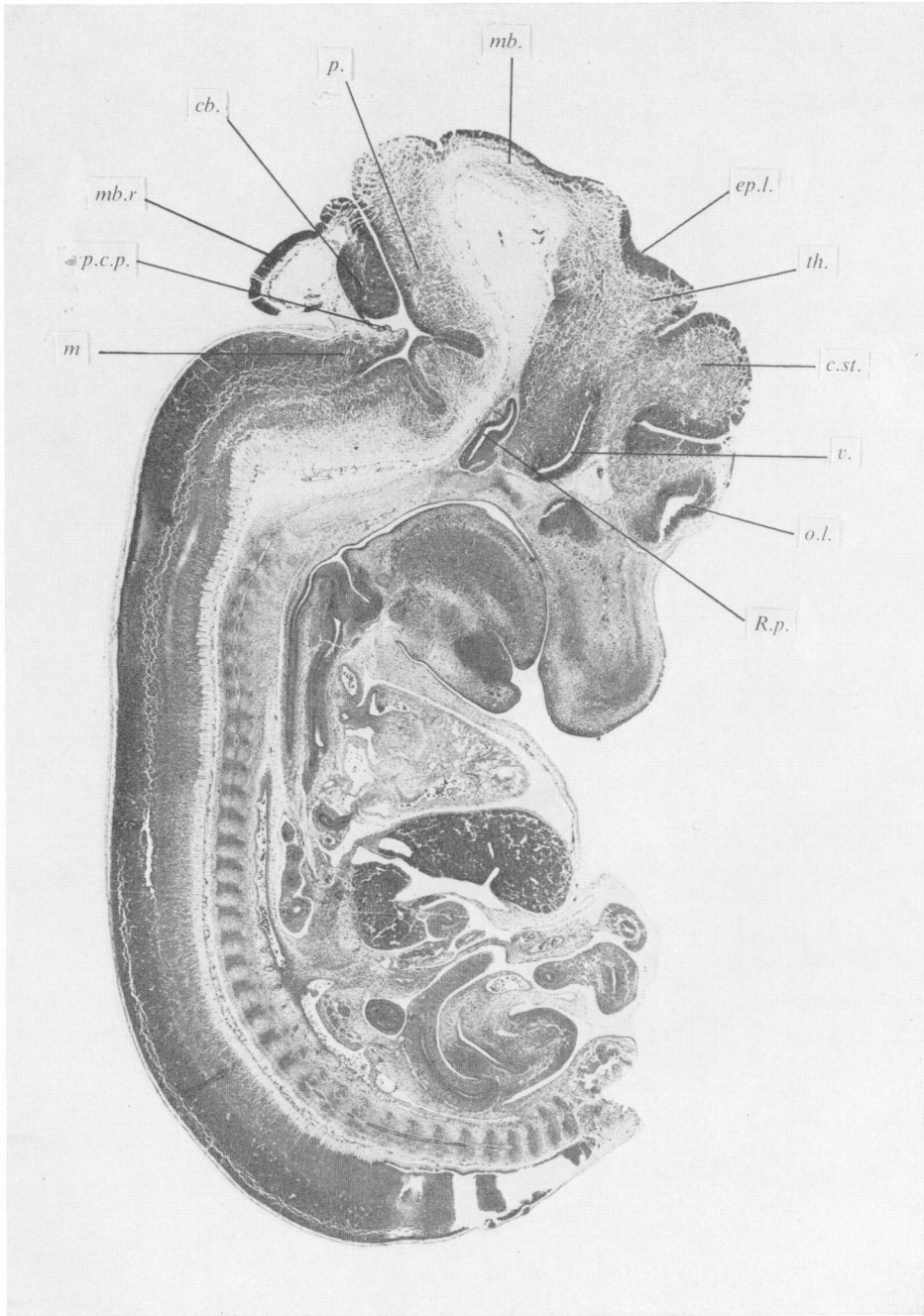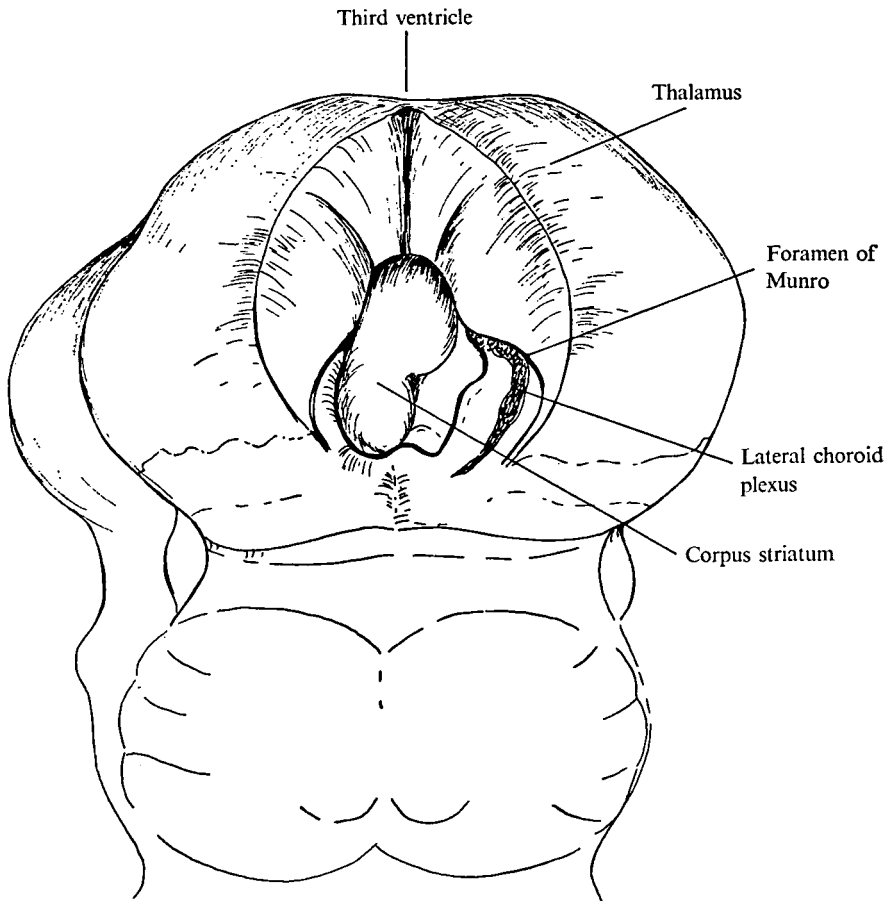Plate II. Exencephalic embryo – 14 days gestation.

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Plate III. Exencephalic embryo – mid-sagittal section – 15 days gestation. *m*, Medulla; *p.c.p.*, posterior choroid plexus; *mb.r.*, midbrain roof; *cb.*, cerebellum; *p.*, pons; *m*:., midbrain; *ep.l.*, ependymal layer; *th.*, thalamus; *c.st.*, corpus striatum; *v.*, third ventricle; *o.l.*, olfactory lobe; *R.p.*, Rathke's pouch.

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opening of the third ventricle is visible as a narrow median slit on the dorsal surface terminating in a tiny central anterior aperture, situated higher up than the opening in the severe form.

(b) Posterior variation

In the most severe form the floor of the medulla and pons is completely exposed (Text-fig. 3). The cerebellum normally forms as a thickening in the lateral walls

Text-fig. 1. Anterior view of exencephalic embryo at 14 days gestation showing severe anterior defect.

of the brain stem immediately posterior to the midbrain and at 15 days has fused across the midline (Rugh, 1968). In the 15-day exencephalic embryo the cerebellum is present as two widely separated masses continuous with the midbrain roof and displaced laterally and ventrally. The fourth ventricle is open to the exterior and the roof plate of the medulla is folded over and fuses posteriorly with the neck ectoderm. The choroid plexus of the fourth ventricle, normally developing in the hind brain roof, begins a small distance from the midline in the

line of fusion between neuroectoderm and neck ectoderm and extends laterally and anteriorly, passing immediately below the cerebellum to enter the open lateral recess of the fourth ventricle (Text-fig. 4).

In the least severe posterior defect the roof of the medulla has closed normally (Plate III). The posterior choroid plexus is situated normally in the fourth
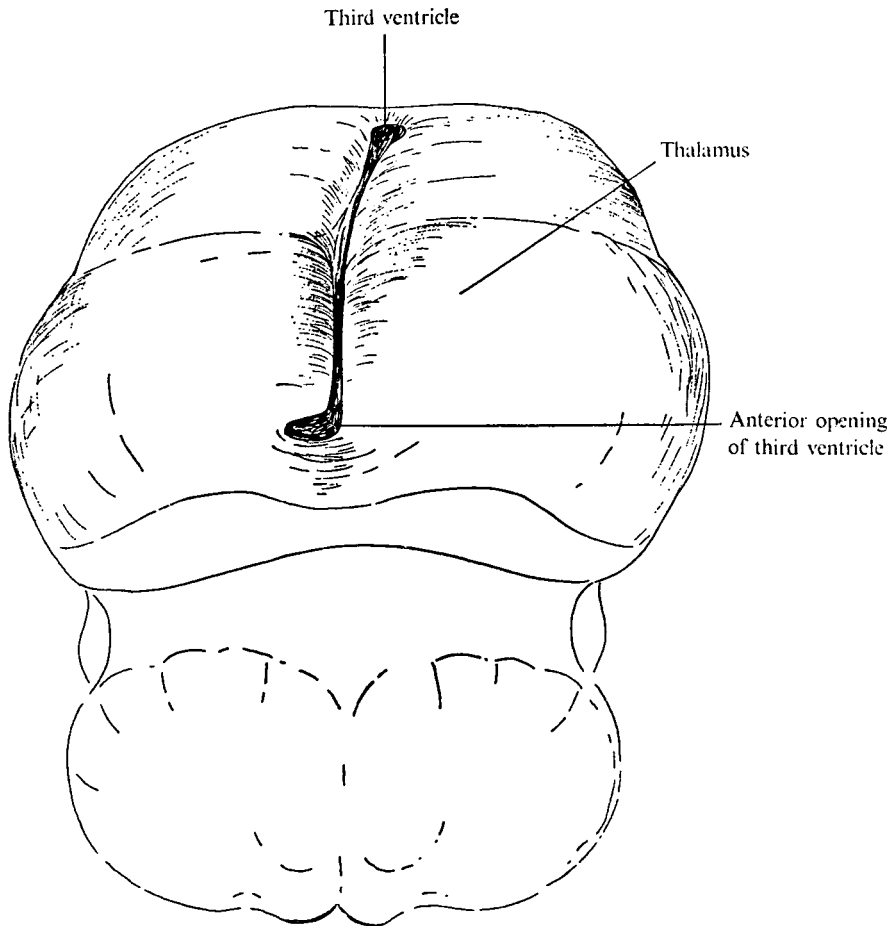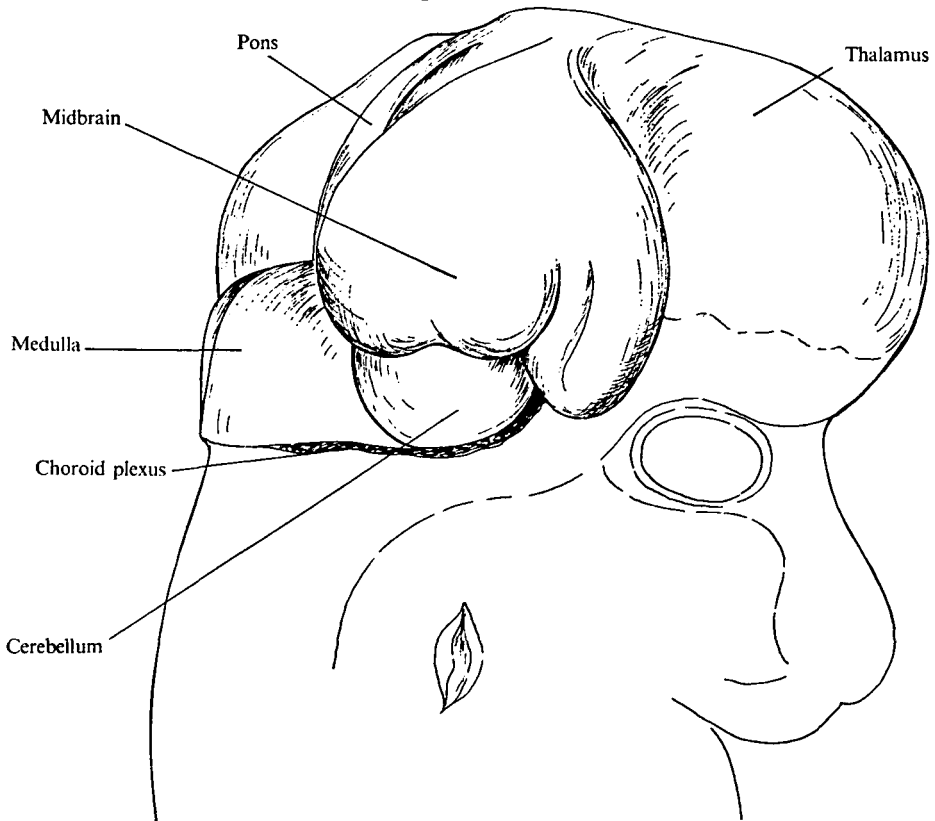

Text-fig. 2. Anterior view of exencephalic embryo at 14 days gestation showing mild anterior defect.

ventricle and in the 15-day embryo the two halves of the cerebellum have fused across the midline. There is only a narrow transverse, slit-like opening in the roof of the fourth ventricle. The out-turned roof of the midbrain has fused laterally with the neck ectoderm and in some embryos the roof of the midbrain has fused across the midline, posterior to the cerebellum and thus covering the roof plate of the hindbrain (Text-fig. 5).

The xn exencephalic mice exhibit all degrees of the anterior and posterior defect between the mild and severe forms described. There is no constant relation-
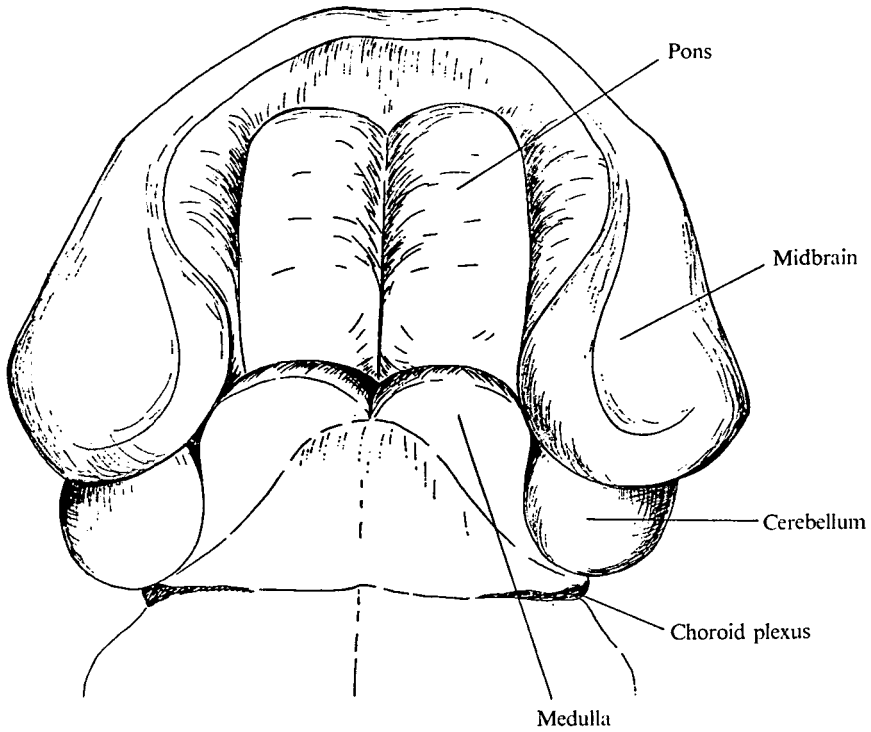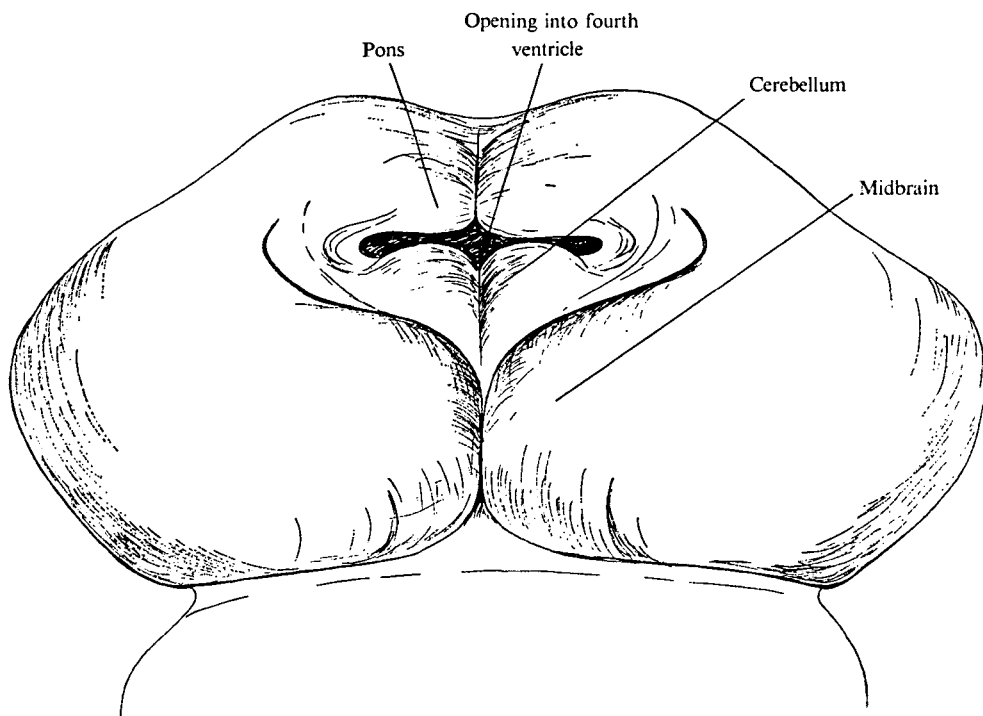
Text-fig. 3. Posterior view of exencephalic embryo at 14 days gestation showing severe posterior defect.

Text-fig. 4. Lateral view of exencephalic embryo at 14 days gestation showing severe posterior defect.

ship between the extent of the anterior and posterior defect; for example a 15-day embryo with the gross anterior opening of the third ventricle showed complete closure of the hindbrain and midline fusion of the cerebellum (Plate III), whereas in a 14-day embryo the hindbrain floor was completely exposed although the anterior opening was small.

Text-fig. 5. Posterior view of exencephalic embryo at 14 days gestation showing mild posterior defect.

5. DISCUSSION

The results show that there is a high probability that exencephaly is due to the partial penetrance of a single recessive gene, xn; that penetrance is higher in females than in males and that it varies greatly (33–84 %) in the genetic milieu studied; and that its expression also varies in a way that affects survival time after birth. It is fully viable until birth. There is no genetic evidence of chromosomal abnormality (cytological preparations have not been made). There are no pleiotropic expressions. These features, taken together, make its inheritance different from that of the four main inherited conditions so far reported.

Morphological studies show variability of expression in detail, and give no support to a supposition of antenatal death. Although exencephalic mice show many similarities with the forms described by Bonnevie (1936) and Snell & Picken (1935), there are major differences. The difference from Bonnevie's is that the telencephalic vesicles are not turned inside out, even in the most severe forms; and Snell's three forms are not described as having the degree of variation

in the anterior and posterior opening observed in the xn mice, nor has a central posterior opening of the fourth ventricle been found in xn mice.

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