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Embryonic development of tetraploid mice during the second half of gestation

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SUMMARY

A small proportion (about 17 %) of experimentally produced tetraploid blastocysts are capable of postimplantation development in the randomly bred Q strain of mice. Four newborn mice, three of which were confirmed as tetraploid, were produced but all were eaten by their mother within a few hours of birth. Studies on the embryonic development of tetraploid mice reveal a variety of developmental abnormalities, especially during the later stages of gestation. At 14½ and 16½ days, tetraploid embryos weigh significantly less than corresponding stage diploids, especially so if litter size is taken into account. Histologically, aberrations are found in many different tissues with a clear hierarchy of susceptibility shown among the organs. For instance, yolk-sac-derived blood, and gonads, are invariably affected and the anterior end of the neural tube also seems to be particularly at risk. Possible explanations for the aberrant development are discussed and it is concluded that strictly genetic reasons can be ruled out and that physiological difficulties imposed by the increased size of tetraploid cells and/or problems produced by lack of cell numbers are instrumental in causing abnormal development. Using weight as a guide it is estimated that tetraploid embryos at 14½ and 16½ days gestation contain about one-quarter as many cells as similar stage diploids.

INTRODUCTION

It has been demonstrated that the random-bred Q strain of mice tolerate tetraploidy to a much greater extent than other mammals (Snow, 1973), although only a small proportion (about 17 %) of tetraploid blastocysts proved capable of sustained development beyond implantation. Harper & Chang (1971) reported similar experiments on early rabbit embryos: after prolonged (18–24 h) exposure to Cytochalasin B (CB) some embryos were capable of developing to the blastocyst stage, and a small proportion would undergo limited development *in utero* after transfer to pseudopregnant foster mothers. Unfortunately these authors did not assess the ploidy of either blastocyst or later embryos. In other studies tetraploid foetal tissue failed to develop (Beatty & Fischberg, 1951, 1952; Beatty, 1957; Astaurov, 1969; Carr, 1971; Graham, 1971; Ingalls & Yamamoto, 1972; and Tarkowski, Witkowska & Opas (in preparation)).

The first attempt to obtain postimplantation tetraploid embryos resulted in

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two morphologically normal embryos at $10\frac{1}{2}$ days gestation (Snow, 1973). Chromosome analysis showed both to be uniformly tetraploid. Attempts were then made to obtain live-born tetraploid young. Four young were born: two spontaneously about 24 h late and two delivered by caesarean section at the normal parturition time. The two naturally born were eaten by their mother and only tissue remnants were retrieved. The two surgically delivered mice were fostered but both were eaten a few hours later. No external abnormalities were observed and the early movements of the newborn seemed normal. Chromosome analysis of tissues (tail tips and forelimb digits) indicated tetraploidy. One of the spontaneously born mice was also tetraploid; it was not possible to determine ploidy in the other.

This report is concerned with the development of tetraploid embryos, particularly during the second half of gestation.

MATERIALS AND METHODS

The randomly bred Q strain of mice was used throughout the study and the procedures for creating tetraploidy were as previously described (Snow, 1973). The mice were kept under a natural diurnal light cycle. Ovulation and mating usually occurred between 24.00 and 03.00 h and gestation is assumed to commence at the later time. Autopsies were always performed between 14.00 and 16.00 h, and embryos are therefore described as being at the $6\frac{1}{2}$-, $14\frac{1}{2}$- and $16\frac{1}{2}$-day stage, etc. At autopsy most embryos and placentae were weighed, after removal of excess surface fluid. Small pieces of tissue were removed from the tail tip and from one forelimb, and along with the foetal membranes these were used to make chromosome preparations according to the method described by Evans, Burtenshaw & Ford (1972) except that culture and colchicine treatment were omitted. The rest of the embryo, and its placenta, was fixed in Sanfelice's fluid, processed for wax embedding, serially sectioned at 6–10 μm either transversely or sagittally and stained with Coles iodine ripened haematoxylin (item 29, Cole 1943) and 0·25% aqueous congo red. This fixative and stain combination has been found to give particularly fine histological definition in mouse embryos. For controls the largest and smallest embryos from four diploid litters of similar gestational age were processed in the same way. Comparison between tetraploids and diploids were made using either photographs or serial camera lucida drawings taken from embryos of comparable developmental stages (assessed by reference to the descriptions given by Gruneberg (1943), Rugh (1968) and Theiler (1972)).

RESULTS

Table 1 summarizes the data derived from 34 experiments in which blastocysts were transferred to pseudopregnant foster mothers. The overall incidence

of implantations is 31·8%: if only those recipients which became pregnant are considered then the incidence rises to 62·8%. These figures represent the lowest and highest estimates of tetraploid blastocyst viability. Table 2 shows the ages, ploidy and sex (where it has been determined) of the embryos. It was not always possible to determine sex chromosomally and the certain histological identification of sex was only possible after $13\frac{1}{2}$ days gestation. The $17\frac{1}{2}$-day embryo was lost in a laboratory accident.

Table 1. *A summary of the development of tetraploid embryos*

Blastocyst cell numbers were determined using Tarkowski's (1966) technique. Blastocysts were transferred into $2\frac{1}{2}$-day pseudopregnant foster mothers (Q strain)

	Preimplantation development			
	2-cell	Blastocysts (%)	Mean cell no.	(No. scored)
CB	2103 ⟶	1082 (51·5)	20·4 ± 0·69	(109)
Control	159 ⟶	138 (86·8)	54·0 ± 2·17	(45)

	Postimplantation development (CB treated only)			
No. transferred	No. recipients	No. pregnant	No. implants*	No. embryos†
846	85	50	269	51

* Includes all sites: embryos, moles and resorptions.
† Includes embryos found dead at autopsy.

The external morphology of all the live 4N embryos except the $17\frac{1}{2}$-day embryo was entirely normal and consistent with their gestational age. The $17\frac{1}{2}$-day embryo had externalized viscera but was otherwise normal. They were however small in comparison with diploids of similar stage, particularly so when litter size is taken into account. Because only a small proportion of 4N blastocysts form embryos they were usually singletons with the occasional litter of two. In diploid mice a reduction in litter size is positively correlated with an increase in foetal and placental weight.

Embryo and placental weights of $14\frac{1}{2}$- and $16\frac{1}{2}$-day 4N embryos have been compared with those of diploids from litters of one or two (Table 3). Foetal but not placental weight is significantly reduced ($P < 0{\cdot}0005$) in tetraploids.

Although up to about 60% of 4N blastocysts initiate implantation, only a few appear to have the potential to form embryos (Tables 1, 2). Histological examinations of sites up to $6\frac{1}{2}$ days gestation shows that the majority of blastocysts develop only as trophoblast giant cells and that few show evidence of an inner cell mass. These observations will be described in detail elsewhere. Where inner cell mass derivatives are present development is not necessarily normal and a high incidence of embryonic mortality is apparent throughout gestation (Table 2).

Table 2. *The postimplantation development of tetraploid blastocysts*

See text for further information

	CN embryos											
Days *p.c.*	4½	5½	6½	8½	9½	10½	12½	13½	14½	16½	17½	term
No. implants	6	14	17	7	5	6	35	24	96	31	8	21
No. embryos	0	0	5	0	2	2	3	4	20	9	2	4
No. live	—	—	2	—	1	2	2	2	15	6	1	2†
Ploidy	—	—	4N	—	4N	4N	4N	1×4N 1×2N/4N	12×4N 3×2N/4N	4×4N 2×2N	?	3×4N 1?
Sex of 4N	—	—	?*	—	?	?	1♂	1♀	4♀	4♀	?	?
	—	—	—	—	—	—	1?	—	4♂	—	—	—
	—	—	—	—	—	—	—	—	4?	—	—	—

* ?=sex not determined.

† Eaten at birth, only remnants retrieved.

Where embryonic development was sustained histological examinations revealed no apparent abnormalities up to $13\frac{1}{2}$ days gestation. In the $14\frac{1}{2}$- and $16\frac{1}{2}$-day embryos, however, a large number of developmental aberrations were found.

Table 4 summarizes the data. No attempt has been made to assess the skeletal normality of the embryos. When an organ is described as small, account has been taken of the reduced size of the tetraploid embryo: the organ is correctly formed with normal substructure. An organ is described as rudimentary if, apart from being small, it is also structurally disorganized. It may be that some

Table 3. *Comparison between the embryo and placental weight of tetraploid mice and singleton diploids at $14\frac{1}{2}$ and $16\frac{1}{2}$ days gestation*

	Embryo and placental weights (mg)			
	$14\frac{1}{2}$ days		$16\frac{1}{2}$ days	
	2N	4N	2N	4N
Embryo	491·2 ± 2·0	192·3 ± 15·1	805·8 ± 15·1	407·6 ± 19·4
placenta	180·3 ± 15·3	145·9 ± 12·4	198·6 ± 20·7	210·3 ± 37·1

of these organs are vestigial in that although normal at earlier stages they have not developed further and may be degenerating. Abnormal organs are generally the correct size but show structural deformities.

The extent to which a foetus was affected is correlated with weight, the smallest showing the severest developmental disturbance. The commonest general defect was the presence of haemorrhages in a variety of tissues, but especially in the lungs (Fig. 3). Interstitial blood, not enclosed in blood vessels, has been found in the stroma of lungs, brain, muscles, testis and spinal cord, as well as free in large quantities in the pericardial cavity of the smallest $14\frac{1}{2}$-day embryo. These haemorrhages are presumably produced by mechanical stresses involved in circulating the large nucleated blood cells derived from the yolk sac (see below), through blood vessels the same size as or smaller than in diploids.

Among the organs studied there is a clear hierarchy of susceptibility to abnormal development. The yolk-sac-derived blood, and the gonads for instance, were always abnormal. The anterior end of the neural tube also seems particularly at risk, with the brain and its diverticula showing irregularities in 10 out of the 12 embryos analysed. Details of the abnormalities in the various organs are as follows.

Blood (Figs. 1, 2). The large size of the nucleated yolk-sac-derived blood cells has already been commented upon. Even allowing for the increase in ploidy these cells are abnormally large, being over four times the volume of corresponding diploid cells. It is likely that the liver-derived erythrocytes are also abnormally large. Although it is not possible to quantitate the difference in size between

Table 4. *A summary of the abnormal development of tetraploid embryos*

Embryonic weight and sex has been included. See text for explanation of symbols

	14½ days								16½ days			
	1	2	3	4	5	6	7	8	1	2	3	4
Sex ...	♀	♂	♀	♂	♀	♀	♂	♂	♀	♀	♀	♀
Weight (mg) ...	139	173	175	179	190	193	248	305	360	407	409	455
Blood, Lung, Gonad	Abnormal in all embryos											
Eye	X	X	X	X	X	X	—	—	X	X	X	—
Optic nerve	—	X	X	O	R	X	—	—	O	O	X	—
Brain	X	X	X	X	X	—	—	—	X	X	X	X
Pineal	O	—	O	O	O	—	—	—	O	O	R	O
Pituitary	—	—	X	X	—	—	—	—	X	—	—	X
Thyroid	R	R	S	S	—	—	—	—	S	S	S	S
Thymus	S	S	—	—	—	—	—	—	S	S	S	S
Pancreas	S	S	—	—	—	—	—	—	S	S	—	S
Kidneys	—	—	—	—	—	—	—	—	X	X	X	X
Adrenals	—	—	—	—	—	—	—	—	X	—	—	—
Spleen, Gut, Heart, Liver, Ears	No abnormalities observed											

X = abnormal, S = small, R = rudimentary, O = absent.

these non-nucleated cells in histological preparations because of their varied shapes, they appear to be more than twice the volume in tetraploids.

Lungs. All embryos contained large blood islands in the lungs (Fig. 3). Regions of necrosis were also found at the extremities of the lobes of the lungs. The organ was often small.

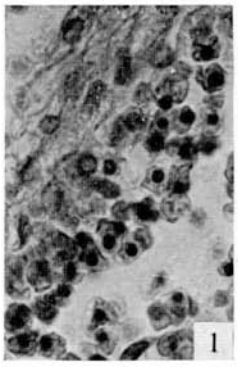
Gonads. Both ovaries and testes were invariably abnormal. Most organs are small and all contained necrotic cells (Figs. 4–8). Germ cells were few, but cell death was not confined to these, considerable areas of stroma also being affected, particularly in ovaries (Fig. 8). The two gonads in an individual may be affected to different degrees.

Eyes and optic nerve. The eyes range in size from normal to very small (Figs. 9, 10); the eye may be rotated in its socket (Fig. 10) and exhibit a variety of abnormalities. The eyes in a single individual are not necessarily affected to the same extent. The most frequent aberration is the severe reduction or absence of the optic nerve (Figs. 11–13). In 14½-day embryo no. 4, one eye possessed an apparently normal optic nerve whereas the other nerve was missing. Other eye abnormalities include an abnormal distribution of the retinal axons (Fig. 14), lens reduction, formation of supernumerary lenses (Fig. 15), and discontinuity in the pigmented retinal epithelium (Fig. 10).

Brain. A number of abnormalities are found in the brain. A general impression gained from serial sagittal sections is that the ventricular space is greatly increased relative to brain size (Figs. 16, 17). This appears to have arisen because of lack of neural tissue in the brain and not simply by ventricular enlargement as a result of, for example, hydrocephaly. The cerebral hemispheres were fused in two embryos, forming a single-chambered structure. The roof of the diencephalon was reduced so that the epiphysis (pineal gland) failed to form in seven embryos (Figs. 17–20). In one 16½-day embryo an unsuccessful attempt had been made to form an epiphysis (Fig. 20). The anterior choroid plexus was sometimes misplaced and oddly shaped. (This may have been brought about by cerebral hemisphere fusion and/or the loss of tissue from the roof of the diencephalon.) The infundibulum failed to develop in two embryos (Figs. 21–23) and was reduced in size in a further two (see also pituitary).

Pituitary. In addition to the infundibular abnormalities described above, two 16½-day embryos showed aberrant development of the anterior lobe components. The pharyngeal diverticulum, Rathke's pocket, had retained its connexion with the pharynx (Fig. 23). (This was associated with a disturbance in the chondrification of the basisphenoid cartilage.) The pituitary component was malformed (Fig. 23).

Kidneys. In the 14½-day embryos kidneys appear to be normal but by 16½ days it is clear that they contain a severely reduced number of glomeruli and tubules. The organs appear empty, with large areas in the medullary region filled with loose parenchymatous tissue. The pelvic epithelium was degenerate in two embryos (Figs. 24, 25).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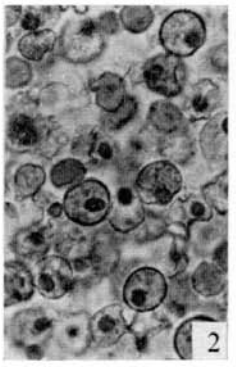
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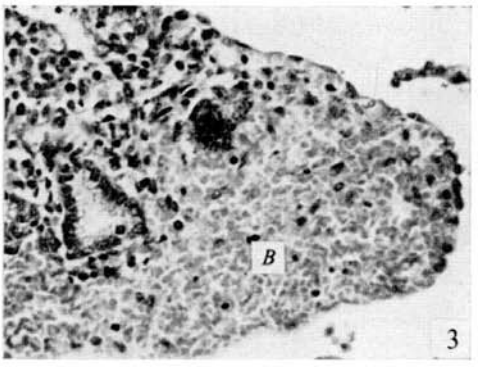

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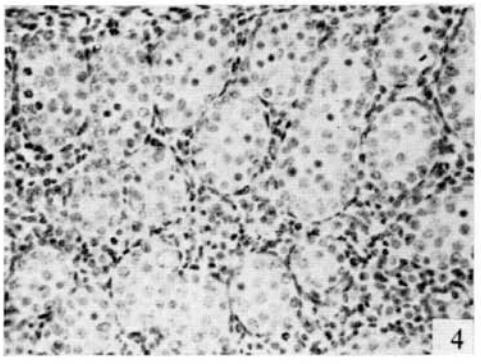
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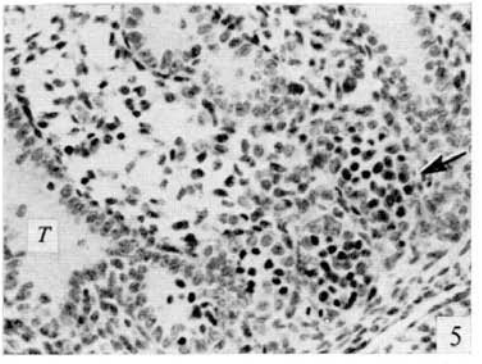

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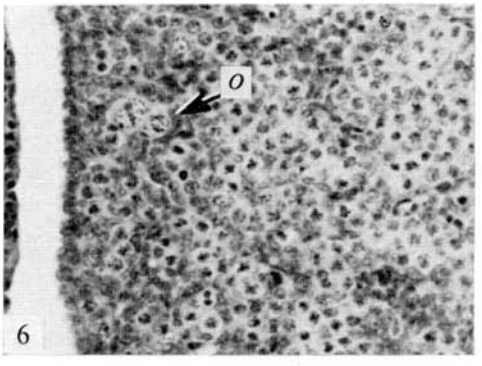

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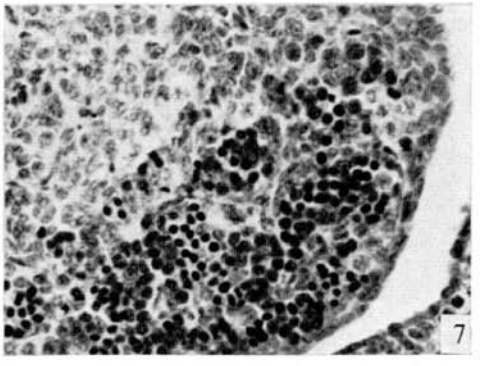
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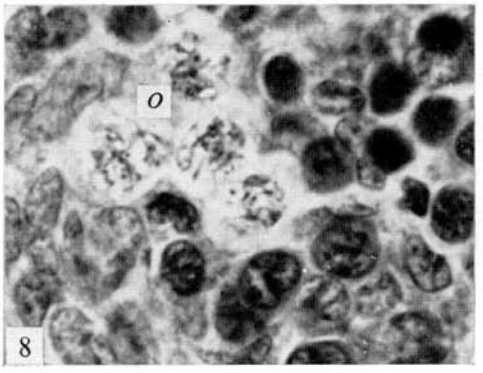

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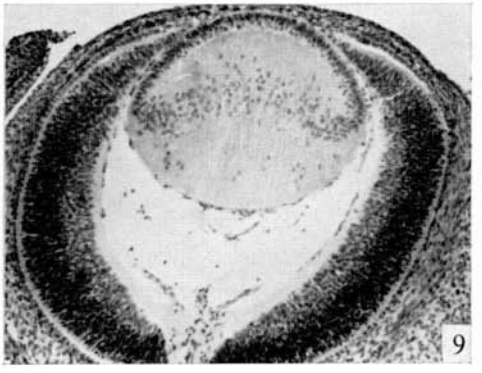
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Adrenal. Only one abnormal adrenal was encountered. This organ was poorly organized; typical adrenal tissue surrounded spaces filled with a loose network of fibroblast-like cells.

DISCUSSION

Table 2 indicates that some mosaic and diploid embryos were obtained from supposedly tetraploid blastocysts. This was not totally unexpected as, although the culture system is designed to minimize the number of cells which revert to diploidy, occasional late-dividing 2-cell embryos could escape detection. The absence of any identifiably mosaic blastocysts among the 109 air-dried examples suggests that they are rare. Furthermore, all the mosaics and diploids occurred in 3 out of the 34 experiments performed. The two diploids and the 14½-day mosaics were produced in two experiments in which the duration of CB treatment was shorter than usual, 8 h instead of the normal 12 h. These were the only experiments in which a short incubation with CB was used; they also produced two 16½-day and three 14½-day embryos which appeared to be uniformly tetraploid when analysed chromosomally and histologically. The other mosaic cannot be similarly accounted for and must be regarded as a chance reversion by one blastomere to the diploid state. If it is assumed that all mosaics developed and were detected, then revertants to diploidy represent less than 0·5% of the recorded implants, and about 0·1% of blastocysts derived from experiments involving incubation with CB of about 12 h.

With regard to the tetraploid embryos it is difficult to envisage a genetic imbalance which might account for the developmental abnormalities unless it is brought about by irregular X-chromosome inactivation. It has been suggested that one active X per two sets of autosomes is the correct balance, and that the impossibility of achieving that ratio in triploids contributes to their developmental problems (Harnden, 1961). In human 69 XXX triploids Barr-body frequency suggests that one inactive X is usual for these cells (reviewed by

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FIGURES 1–9

Fig. 1. 2N 14½-day embryo. Nucleated yolk-sac-derived blood in the auricle. × 800.

Fig. 2. 4N 14½-day embryo. As Fig. 1.

Fig. 3. 4N 16½-day embryo. Extensive blood island (*B*) in a lobe of the lung. × 320.

Fig. 4. 2N 14½-day embryo. Testis. × 320.

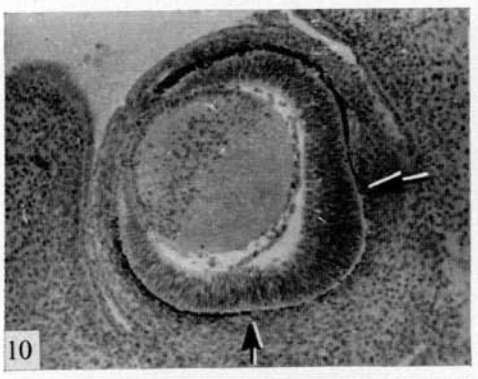
Fig. 5. 4N 14½-day embryo. Testis. Note empty tubules (*T*) and pycnotic nuclei (arrow). × 320.

Fig. 6. 2N 14½-day embryo. Ovary (*O* = oocytes). × 320.

Fig. 7. 4N 14½-day embryo. Ovary. This particular organ was very small and contained many pycnotic nuclei. × 320.

Fig. 8. 4N 14½-day embryo. Ovary. Oocytes (*O*) are found occasionally. The pycnotic nuclei seen here appear to be in stromal cells. × 2000.

Fig. 9. 2N 14½-day embryo. Eye. × 125.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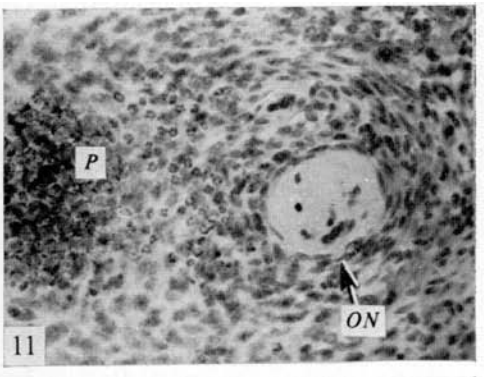

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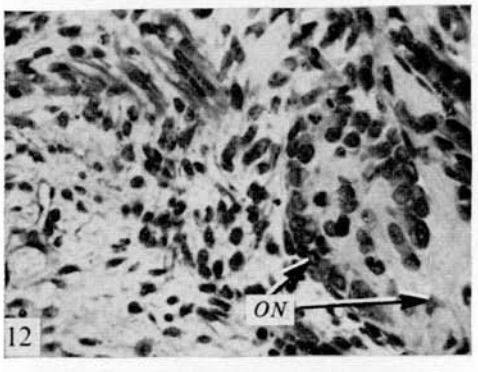

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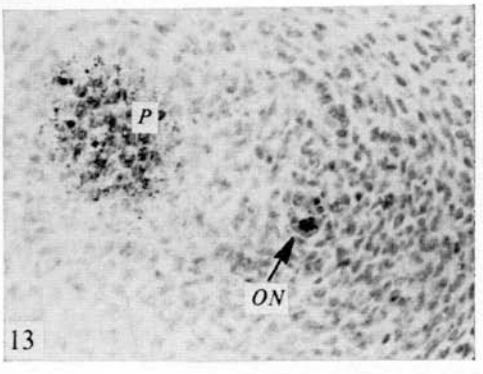

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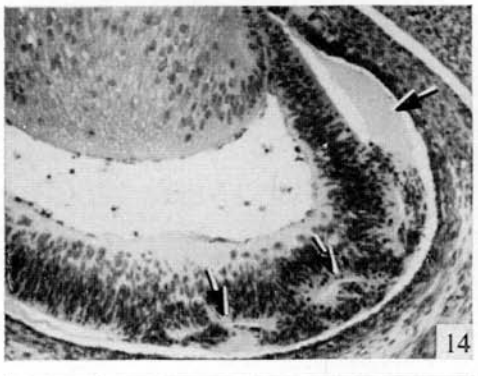
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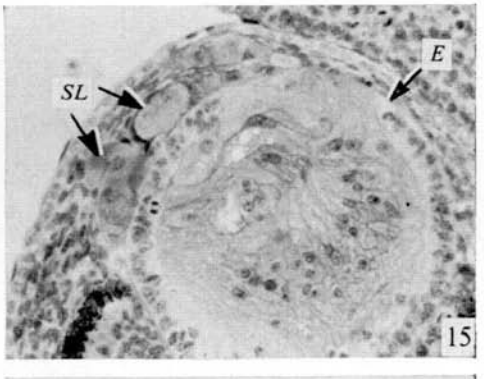

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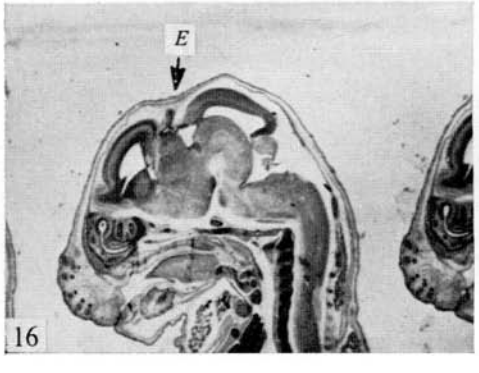

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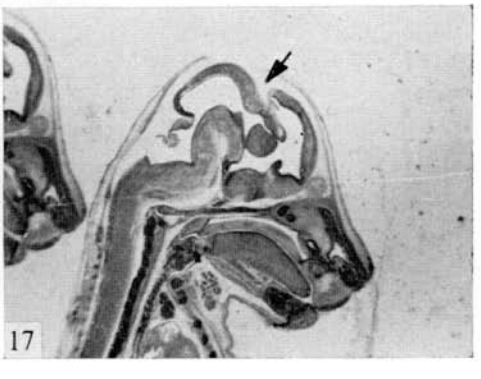
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Niebuhr, 1974), but that is in contradiction to the single autoradiographic study (Niebuhr, Sparrevohn, Henningsen & Mikkelsen, 1972) in which 59% of cells showed two late-replicating, and presumably inactive, X-chromosomes. Late replication is regarded as a more reliable guide to chromosome inactivity (Cooke, Black & Curtis, 1972) than is the very variable Barr-body frequency (Berkeley & Faed, 1970; Blackston & Chen, 1972). Whether one active X chromosome per cell, regardless of ploidy, could be the rule remains to be investigated. In tetraploids such a system would yield an unbalanced genome.

Bearing in mind that in all but three tissues normal development of each organ system has been observed in at least three tetraploid embryos, and remembering that the lung abnormalities may be a secondary effect of the abnormal yolk-sac blood, strictly genetic effects, whether brought about by chromosome inactivation or some other means, can probably be ruled out. It seems much more likely that the developmental problems encountered by tetraploids are physiological and numerological in nature. For instance it has already been noted (Snow, 1973) that the cells in tetraploids are larger than in diploids. If tetraploid cells are twice the volume of diploid cells – as they undoubtedly are at the 2–4-cell stage when they are created, and which might be expected from doubling the genome of the cell – then the surface area of each cell, assuming it to be a sphere, increases only 59%. This circumstance might prove limiting with regard to the passage in and out of the cell of metabolites and waste products, etc. In order to restore the surface-to-volume ratio in a tetraploid cell, if that is what is important, considerable changes in shape would be necessary which would probably result in morphological deformity. Evidence

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FIGURES 10–17

Fig. 10. 4N 14½-day embryo. Eye. The eye is small, rotated in its socket and the pigmented retinal epithelium is discontinuous (arrow). × 125.

Fig. 11. 4N 14½-day embryo. Normal optic nerve (*ON*). The pigmented epithelium at the back of the eye is visible (*P*). × 320.

Fig. 12. 4N 14½-day embryo. Reduced and disorganized optic nerve (*ON*). × 800.

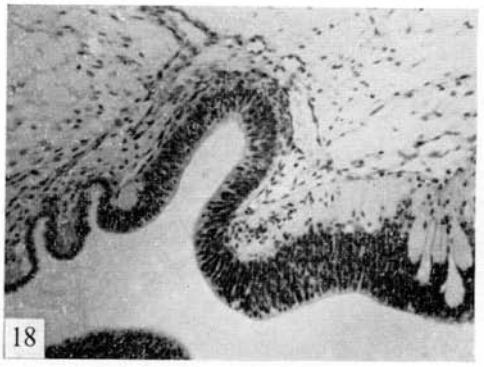
Fig. 13. 4N 14½-day embryo. Optic nerve absent. *ON* marks the last remnant of the optic stalk. The presence of a few pigment granules indicates an extension from the pigmented epithelium (*P*). × 320.

Fig. 14. 4N 16½-day embryo. Eye. The retinal axons (arrow) are abnormally distributed: compare with Fig. 9. × 125.

Fig. 15. 4N 16½-day embryo. Eye. Small supernumerary lenses (*SL*) have formed. The lens itself is malformed and its epithelium (*E*) appears to be discontinuous. × 320.

Fig. 16. 2N 16½-day embryo. Sagittal section through head. Note proportion of tissue and ventricles in the brain. See also the pineal (*E*).

Fig. 17. 4N 16½-day embryo. Sagittal section through the head. Note the altered proportions of neural tissue and ventricles in the brain. In this animal the roof of the diencephalon is lacking the pineal region (arrow).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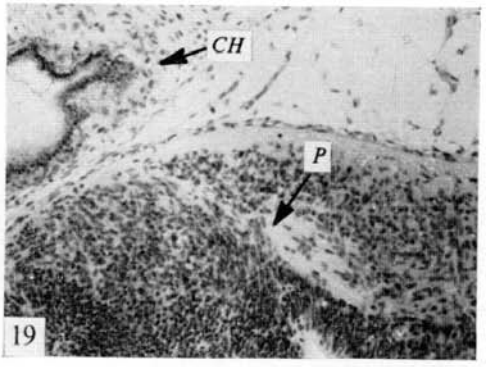

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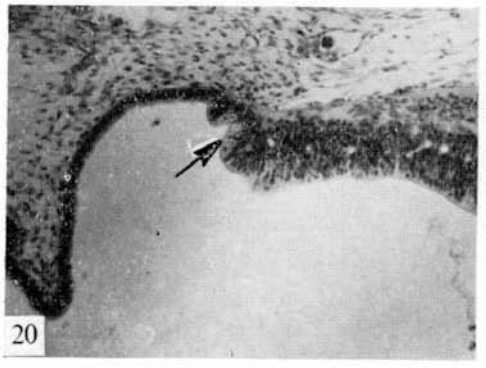
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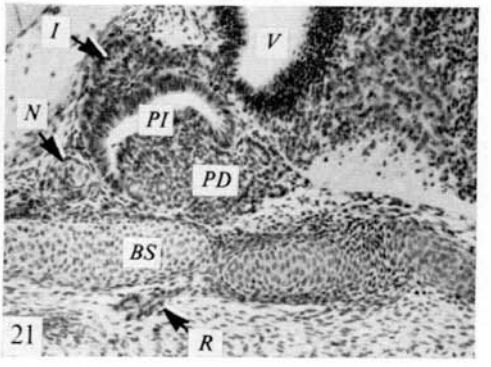

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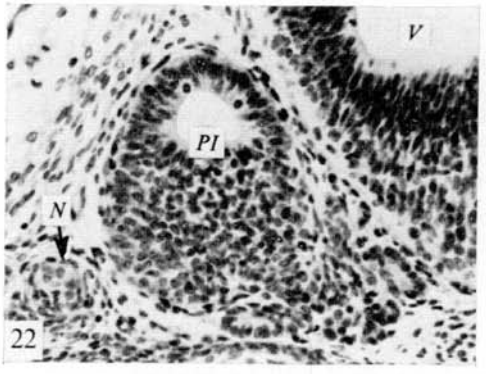

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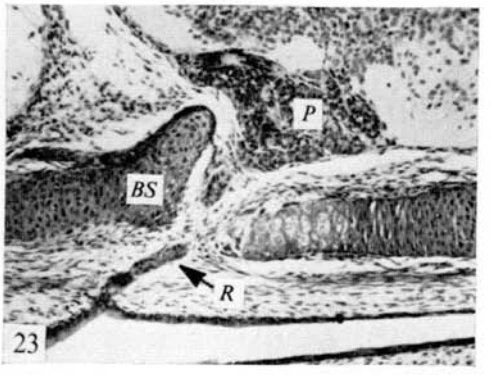

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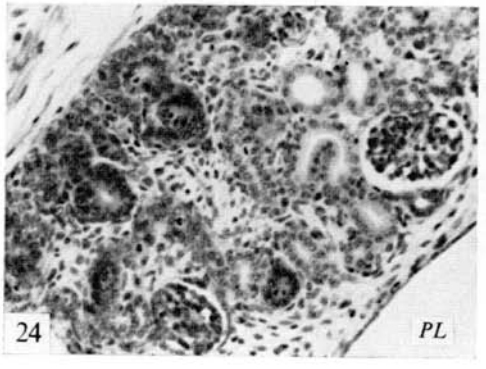

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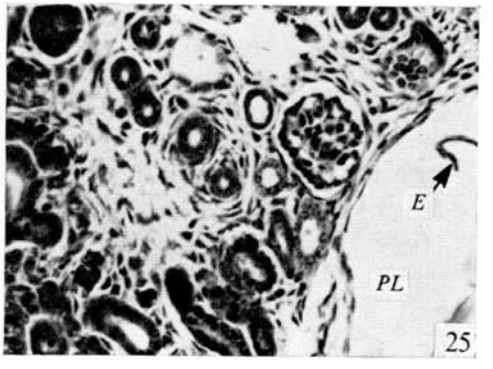

25

from other species which tolerate tetraploidy is unhelpful in this context. In Amphibia, where a complete ploidy series from haploid to hexaploid is known, cell size increases proportionately with ploidy and yet normal development to adulthood is achieved in all classes (Frankhauser, 1945). In these species, therefore, any physiological problems associated with cell size are overcome. In Cyprinid fish on the other hand both diploid and tetraploid species exist, and the cell size in these appears to be of fundamental importance in that in tetraploids the diploid cell size has been retained (Schmidtke & Engel, 1975). Furthermore, the cell-size control has been achieved without a concomitant reduction in ribosomal genes (Schmidtke, Zenzes, Dittes & Engel, 1975) such as has been reported in other organisms in which cell size has changed (Pederson, 1971; Siegel, Lightfoot, Ward & Keener, 1973; Maher & Fox, 1973).

Tetraploid blastocysts contain on average 38% as many cells as diploids (Table 1). Analysis of the early postimplantation period suggests that this paucity of cells results in many cases in the failure to form a competent inner cell mass in the blastocyst (Snow, in preparation). Perhaps during later embryogenesis the reduction in cell numbers interferes with the development of some organ systems because there are not sufficient cells to form a functional primordium. This could explain the smallness of many organs and the absence of a large part of the diencephalon in many tetraploid embryos. A crude estimate of the cell-number reduction may be made in the following manner. Tetraploid embryos are about half the weight of diploids (Table 3) and cells are about twice the volume. Therefore providing the density of tissue is similar in both cases, the 4N embryos at 14½ and 16½ days contain about one-quarter

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FIGURES 18–25

Fig. 18. 4N 14½-day embryo. Normal pineal gland. × 125.

Fig. 19. 4N 14½-day embryo. Pineal gland absent. The stratification of the cells in this region (*P*) probably indicate where it should have formed. The cerebral hemispheres (*CH*) were also abnormal in this embryo. × 125.

Fig. 20. 4N 14½-day embryo. Abnormal pineal. An attempt to form a diverticulum has been made (arrow). × 125.

Fig. 21. 4N 14½-day embryo. Normal pituitary gland. *BS*=basispheroid cartilage; *I*=infundibulum; *PI*, *PD*=pars intermedia and distalis; *N*=end of notochord; *R*=rudiment of Rathke's pocket (see Fig. 23); *V*=ventricle of brain. × 125.

Fig. 22. 4N 14½-day embryo. Pituitary. Infundibulum is absent and the pars intermedia (derived from Rathke's pocket) is all that is present. × 800.

Fig. 23. 4N 16½-day embryo. Abnormal pituitary. Infundibulum absent, basisphenoid cartilage (*BS*) abnormal and the pharyngeal diverticulum, Rathke's pocket (*R*) persists. The distal end of Rathke's pocket (*P*) has failed to form a normal pars intermedia. × 125.

Fig. 24. 2N 16½-day embryo. Kidney. *PL*=lumen of pelvis. × 320.

Fig. 25. 4N 16½-day embryo. Kidney. Note excessive interstitial spaces in stroma and the degenerate pelvic epithelium (*E*). × 320.

as many cells. Crude though it is, even this estimate may be too *large*, as histological sections show a greater proportion of interstitial and coelomic space in tetraploids in addition to the already noted reduction in brain neural tissue. Consequently a greater volume of extracellular fluid is contributing to the weight of tetraploid embryos.

One consequence of reduced tissue volume is that whatever the parameters operative in pattern formation they are required to act over smaller distances than usual in order to achieve normal development. Severe reduction in tissue volume could impose insurmountable problems in such regulative processes.

Whatever difficulties are imposed upon development by low cell numbers it is clear that this explanation cannot be invoked to account for the malformation associated with the eye and optic nerve. The supernumerary lenses and retinal disorganization are true deformities, as the tissue concerned is manifestly present. Similarly, by the very nature of eye formation the connexion between optic cup and midbrain must have existed, and the absence of an optic stalk is therefore secondary degeneration. Possible mechanisms whereby these malformations may be produced are obscure. Very similar abnormalities have been reported in rodents, especially rats, resulting from X-irradiation, administration of trypan blue, anti-mitotic agents and actinomycin D, as well as vitamin deficiencies and unbalanced thyroid activity (see Tuchmann-Duplessis & Mercier-Parot, 1961).

It is also interesting to note that in trisomy 19 in the mouse (White, Tjio, Van de Water & Crandall, 1974) some of the abnormalities described are similar to those occurring in tetraploid embryos. For instance foetal weight was reduced, and extensive degeneration of ovarian tissue occurred. Testes were unaffected. Unpublished data (White, personal communication) also suggests that brain and lung weights are significantly reduced in trisomy 19 mice. White, *et al.* (1974) postulate that the tissue reductions in their trisomic mice are the result of decreased cell duplication rate and a deficiency in cell numbers.

REFERENCES

ASTAUROV, B. L. (1969). Experimental polyploidy in animals. *A. Rev. Genet.* **3**, 99–126.

BEATTY, R. A. (1957). *Parthenogenesis and Polyploidy in Mammalian Development.* London: Cambridge University Press.

BEATTY, R. A. & FISCHBERG, M. (1951). Heteroploidy in mammals. I. Spontaneous heteroploidy in pre-implantation mouse eggs. *J. Genet.* **50**, 345–359.

BEATTY, R. A. & FISCHBERG, M. (1952). Heteroploidy in mammals. III. Induction in pre-implantation mouse eggs. *J. Genet.* **50**, 471–479.

BERKELEY, M. I. K. & FAED, M. J. W. (1970). A female with the 48 XXXX karyotype. *J. med. Genet.* **7**, 83–85.

BLACKSTON, R. D. & CHEN, A. T. L. (1972). A case of 48 XXXX female with normal intelligence. *J. med. Genet.* **9**, 230–249.

CARR, D. H. (1971). Chromosome studies in selected spontaneous abortions. Polyploidy in man. *J. med. Genet.* **8**, 164–174.

COLE, E. C. (1943). Studies on haematoxylin stains. *Stain Technol.* **18**, 125–142.

COOKE, P., BLACK, J. A. & CURTIS, D. J. (1972). Comparative clinical studies and X-chromosome behaviour in a case of XXXX/XXXXX mosaicism. *J. med. Genet.* **9**, 235–238.

EVANS, E. P., BURTENSHAW, M. D. & FORD, C. E. (1972). Chromosomes of mouse embryos and newborn young: preparations from membrane and tail tips. *Stain Technol.* **47**, 229–235.

FRANKHAUSER, G. (1945). The effect of changes in chromosome number on amphibian development. *Q. Rev. Biol.* **20**, 20–78.

GRAHAM, C. F. (1971). Virus assisted fusion of embryonic cells. In 'In vitro *Methods in Reproductive Cell Biology*', *3rd Karolinska Symposium* (ed. E. Diczfalusy). Stockholm: Karolinska Institute.

GRUNEBERG, H. (1943). The development of some external features in mouse embryos. *J. Hered.* **34**, 89–92.

HARNDEN, D. G. (1961). Nuclear sex in triploid XXY human cells. *Lancet* **2**, 488.

HARPER, M. J. K. & CHANG, M. C. (1971). Some aspects of the biology of mammalian eggs and spermatozoa. *Adv. reprod. Physiol.* **5**, 167–218.

INGALLS, T. H. & YAMAMOTO, M. (1972). Hypoxia as a chromosomal mutagen. Triploidy and tetraploidy in hamster embryos. *Arch. Environ. Health* **24**, 305–315.

MAHER, E. P. & FOX, D. P. (1973). Multiplicity of ribosomal RNA genes in *Vicia* species with different nuclear DNA contents. *Nature New Biol.* **245**, 170–172.

NIEBUHR, E. (1974). Triploidy in man: cytogenetical and clinical aspects. *Humangenetik* **21**, 103–125.

NIEBUHR, E., SPARREVOHN, S., HENNINGSEN, K. & MIKKELSEN, M. (1972). A case of live born triploidy (69 XXX). *Acta paediat., Scand.* **61**, 203–208.

PEDERSON, R. A. (1971). DNA content, ribosomal gene multiplicity and cell size in fish. *J. exp. Zool.* **177**, 65–78.

RUGH, R. (1968). *The Mouse: its Reproduction and Development.* Minneapolis: Burgess Publ. Co.

SCHMIDTKE, J. & ENGEL, W. (1975). *Biochemical Genetics* (in the Press).

SCHMIDTKE, J., ZENZES, M. T., DITTES, H. & ENGEL, W. (1975). Regulation of cell size in fish of tetraploid origin. *Nature, Lond.* **254**, 426–427.

SIEGEL, A., LIGHTFOOT, D., WARD, O. C. & KEENER, S. (1973). DNA complementary to ribosomal RNA: relation between genomic proportion and ploidy. *Science, N.Y.* **179**, 682–683.

SNOW, M. H. L. (1973). Tetraploid mouse embryos produced by cytochalasin B during cleavage. *Nature, Lond.* **244**, 513–515.

TARKOWSKI, A. K. (1966). An air-drying method for chromosome preparation from mouse eggs. *Cytogenetics* **5**, 394–400.

THEILER, K. (1972). *The House Mouse.* Berlin: Springer.

TUCHMANN-DUPLESSIS, H. & MERCIER-PAROT, L. (1961). Production of congenital eye malformations, particularly in rat fetuses. In *The Structure of the Eye* (ed. G. Smelser). New York: Academic Press.

WHITE, B. J., TJIO, J. H., VAN DE WATER, L. C. & CRANDALL, C. (1974). Trisomy 19 in the laboratory mouse: II. Intra-uterine growth and histological studies of trisomics and their normal littermates. *Cytogenet. Cell Genet.* **13**, 232–245.

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