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Clinical details, cytogenetic studies, and cellular physiology of a 69,XXX fetus, with comments on the biological effect of triploidy in man

CHRISTINE M. GOSDEN, M. O. WRIGHT,* W. G. PATERSON, and K. A. GRANT

From the MRC Clinical and Population Cytogenetics Unit, Western General Hospital; and the Obstetric Department, Eastern General Hospital, Edinburgh

Summary. A triploid fetus, 69,XXX, aborted spontaneously at 26 weeks' gestation. It had multiple abnormalities including syndactyly of the hands and feet, single palmar creases, hypoplasia of the adrenals and ovaries, hypertrophy of thigh muscles, and abnormalities of the brain. The placenta was large and showed hydatidiform degeneration. The pregnancy had been complicated by acute dyspnoea, pre-eclampsia, and postpartum haemorrhage.

Detailed cytogenetic studies, using banding and fluorescence techniques, were performed on fetus and parents. Meiotic studies were made on the fetal ovaries. Muscle cell differentiation and electrophysiological relationships of cultured skin fibroblasts were examined in an attempt to study the way in which the extra haploid set of chromosomes exerts its effect on the phenotype.

The antenatal diagnosis of late triploidy is discussed.

The finding that 25 per cent of late triploids have spina bifida is further evidence that meningomyelocele has a genetic component and strongly suggests that this results from chromosomal imbalance or a regulatory gene disturbance.

Only recently has the pure triploid state been recognized in man. Penrose and Delhanty in 1961 described an early spontaneous triploid abortion. Beischer *et al* in 1967 reported a late triploid abortion, and in the same year Bernard *et al* and Edwards *et al* described triploid infants surviving only a few hours. Diploid/triploid mosaics may survive much longer but will not be considered in this report. To date 22 cases of triploidy over 24 weeks have been published and these, with 3 further cases, are summarized in Table I.

In some vertebrates polyploidy is well tolerated, and yet in man this imbalance is lethal. The mechanism by which an extra haploid set of chromosomes exerts its abnormal effects is not understood. In this paper we consider in detail the pregnancy and some of the physiological and biological features of one of our cases and comment on the variable expression of the congenital defects.

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* Present address: Department of Physiology, University of Edinburgh.

Case report

The patient and her husband were Scottish, aged 22 and 24. The first pregnancy in 1971 ended with delivery of a premature normal male. Oral contraception begun thereafter was discontinued in January 1972. At 10 weeks' gestation on 20 December 1972, there was no oedema or proteinuria and the blood pressure was 116/60 mmHg. Fetal movements were first felt at 16 weeks. Two weeks later proteinuria was present but there was no oedema and the blood pressure was only 150/80 mmHg. At 19 weeks' gestation she complained of acute breathlessness, wheeze, and swelling of the legs. The blood pressure was 132/85 mmHg. She was given frusemide 40 mg each morning and her symptoms quickly cleared. By the 21st week she felt well and no abnormalities were found except a blood pressure of 150/88 mmHg. Frusemide was discontinued. Four weeks later the blood pressure was 160/100 mmHg but

TABLE I

Author and Year	Recent OC	PET	Hydram.	Resp. Illness	Oestriol (μ mol/24h)	Weight (kg)	Sex	Gestation (w)
Beischer *et al*, 1967	?	Severe	Yes	Yes	5.2	0.35	XXX	24
Bernard *et al*, 1967	?	No	No	No	None	1.8	XXY	32
Edwards *et al*, 1967	?	No	No	Yes	None	1.7	XXY	32
Papiernik-Berkhauer, 1968	No	No	No	No	None	1.4	XXY	42
Butler *et al*, 1969	?	No	No	No	None	1.8	XXX	35
Keutel, Dollman, and Munster, 1970	No	?	Yes	?	?	1.9	XXY	36
Leisti *et al*, 1970	?	?	?	?	?	2.8	XXY	41
Schindler and Mikamo, 1970	?	Mild	Yes	No	None	1.5	XXY	39
Paterson *et al*, 1971	No	Severe	Yes	No	None	1.6	XXX	29
	No	Severe	?	No	None	0.789	XXX	25
Prats *et al*, 1971	?	No	No	No	None	2.4	XXY	40
Schmickel *et al*, 1971	?	?	No	No	None	2.5	XXY	39
Finley *et al*, 1972	No	?	?	?	?	2.2	XXY	?40
Niebuhr *et al*, 1972	No	?	?	?	Low	1.6	XXX	37
Simpson *et al*, 1972	No	?	?	?	?	2.5	XXY	40
Uchida and Lin, 1972	No	?	?	?	?	?	XXY	27
Wertelecki *et al*, 1972	?	?	?	?	?	?	XXX	?
Halbrecht *et al*, 1973	No	No	No	Asthma	None	1.0	XXX	39
Hashimoto *et al*, 1973	?	Yes	?	?	?	0.771	XXY	25
Walker *et al*, 1973	?	Oedema	No	No	None	2.6	XXY	38
	No	Oedema	Yes	No	43.4	3.5	XXX	36
	No	mild	Yes	Yes	12.1	1.9	XXY	36
Gosden *et al*, 1974*	No	Mild	Yes	No	None	1.5	XXX	34
	No	Mild	Yes	Yes	29.8	0.86	XXX	26
A. B. Fulton and D. M. Albert, 1975 personal communication	No	No	No	No	None	0.95	XXY	30

* Case reported in detail.

Explanation of numbers and abbreviations recorded under fetal abnormalities in Table I.

Numbers: 1: Heart defects; 2: kidney defects; 3: hypoplastic gonads; 4: hypoplastic adrenals; 5: coloboma; 6: abnormal ears; 7: wine bottle thighs; 8: central nervous system abnormalities including hydrocephalus; 9: spina bifida, meningomyelocele; 10: genital abnormalities; 11: syndactyly of fingers; 12: syndactyly of toes; 13: harelip; 14: red fetus; 15: exomphalos.

Abbreviations: Recent OC, oral contraception in previous 6 months; PET, pre-eclamptic toxaemia; hydram., hydramnios; resp. illness, respiratory illness; PPH, post-partum haemorrhage.

there was no oedema or proteinuria. She was admitted to hospital because of hypertension. Urinary oestriol excretion was 29.8 μmol/24h. Four days later transient proteinuria (3g/24h) and polyhydramnios appeared. She went into spontaneous labour at 26 weeks and during labour the blood pressure rose to 170/130 mmHg and was difficult to control. After 7 hours an abnormal female was born who died within 1 hour. An atonic postpartum haemorrhage of 1 litre occurred with delivery of the placenta. She became pregnant again in December 1973 and was delivered at term of a normal male.

Pathology of fetus and placenta

The fetus was dark red in colour and weighed 0.86 kg. The placenta weighed 0.75 kg and showed diffuse hydatidiform degeneration (Fig. 1 and 2).

The skull vault was large, with a prominent forehead and the nasal bridge was depressed. The ears were low-set and malformed. The upper lip was long and intact. The oral cavity, tongue, and palate were normal. The ribcage was flared with wide-set aplastic nipples. There were flexion deformities of the fingers, syndactyly of the third and fourth digits, hyperconvex nails, and bilateral singular palmar creases. In the lower limbs overdevelopment of the thigh muscles gave rise to a 'wine bottle' shaped appearance. A right talipes equinovarus was present and there was bilateral syndactyly of the third and fourth toes with wide first interdigital spaces.

The heart was grossly enlarged with thickened musculature of both left and right ventricles. There were no septal defects. The lungs were small and haemorrhagic. The gastrointestinal tract was normal and was contained within the abdomen. The right kidney was normal but the left was entirely cystic. The adrenal glands and both ovaries were hypoplastic.

The brain was above average size for the period of gestation but the stage of cortical development was normal. The parieto-occipital cortex was thin and had collapsed inwards bilaterally. The median fissure was ill developed between the frontal poles which were partly fused. An abnormal fissure ran horizontally round the frontal poles at the junction of the lower and middle thirds ending posteriorly on either side in front of the optic chiasma. The accessory lobes were of appreciable size and had a smooth surface (Fig. 3). Both lateral and third ventricles were dilated and the septum pellucidum was absent. The ependyma was thickened and nodular with numerous subependymal petechial haemorrhages (Fig. 4). The hind brain showed a Dandy Walker abnormality.

On light and electron microscopy heart muscle fibres looked normal except for nuclear enlargement. Microscopy of the ependymal nodules showed that they were foci of neuroblasts. Some nodules contained haemosiderin granules, indicative of earlier haemorrhage.

Materials and methods

Blood culture

Karyotyping of the fetus was performed on both blood leucocytes and skin fibroblasts. The parents were also karotyped from peripheral blood leucocytes. Blood

Alive (h)	Placenta (kg)	Hydatidiform Degeneration	PPH	Fetal Abnormalities
SB	?	Yes	No	2, 7, 8, 9
15	?	?	No	6, 8, 10, 11, 12
?10	Large	Yes	No	5, 6, 8, 10
SB	0.26	No	No	1, 2, 6, 10, 11
23	0.935	No	No	1, 2, 3, 4, 5, 6, 8, 10
0.25	1.7	Yes	?	8, 9, 11, 13
168	1.4	?	?	2, 6, 10, 11, 12
6	0.88	Yes	No	2, 4, 5, 6, 7, 10, 11
SB	0.817	Yes	Yes	7, 11, 13, 14, 15
SB	0.231	Yes	No	11, 14, 15
36	Large	Yes	No	2, 4, 6, 7, 8, 9, 10, 12
2	Large	?	No	5, 6, 8, 9, 10, 11, 12
11	?	?	?	1, 6, 13
93	?	?	?	4, 6, 8, 11
10	?	?	?	1, 5, 6, 7, 8, 9, 10
4	?	?	?	1, 8, 10, 13
?	?	?	?	No data given
SB	?	?	?	1, 8
SB	?	?	?	15
SB	?	?	No	1, 2, 3, 4, 8, 10, 13
8	1.6	Yes	Yes	1, 12
7	0.83	Yes	?	5, 8, 10, 12
7	0.6	No	Yes	10, 11, 12, 14
1	0.75	Yes	Yes	1, 2, 3, 4, 6, 8, 11, 12, 14
SB	?	No	No	3, 5, 6, 8, 9, 10, 12, 13

was obtained from the fetus by cardiac puncture and cultured by the micro method modified from that of Hungerford (1965) described by Evans, Buckton, and Sumner (1971) for peripheral leucocytes. Orcein-stained metaphase preparations were prepared for total chromosome counts, individual chromosomes were identified using the acetic/saline/giemsa (ASG) technique of Sumner, Evans, and Buckland (1971), and fluorescent polymorphisms in parents and fetus were examined using quinacrine fluorescence.

Cell culture

Cell cultures obtained from fetal skin and muscle biopsies (*post mortem*) were prepared by the method of Gosden and Emery (1973). The explants were placed in either glass or plastic bottles, covered with Hams F10 growth medium, supplemented with 10% fetal calf serum and antibiotics, and incubated at 37°C in a 10% CO_2/air mixture. After 12 days' growth in primary culture, cells were trypsinized into larger glass bottles or Falcon plastic flasks. The culture bottles were injected with Colcemid 4 hours before harvesting for cytology. Slide preparations were made for staining:

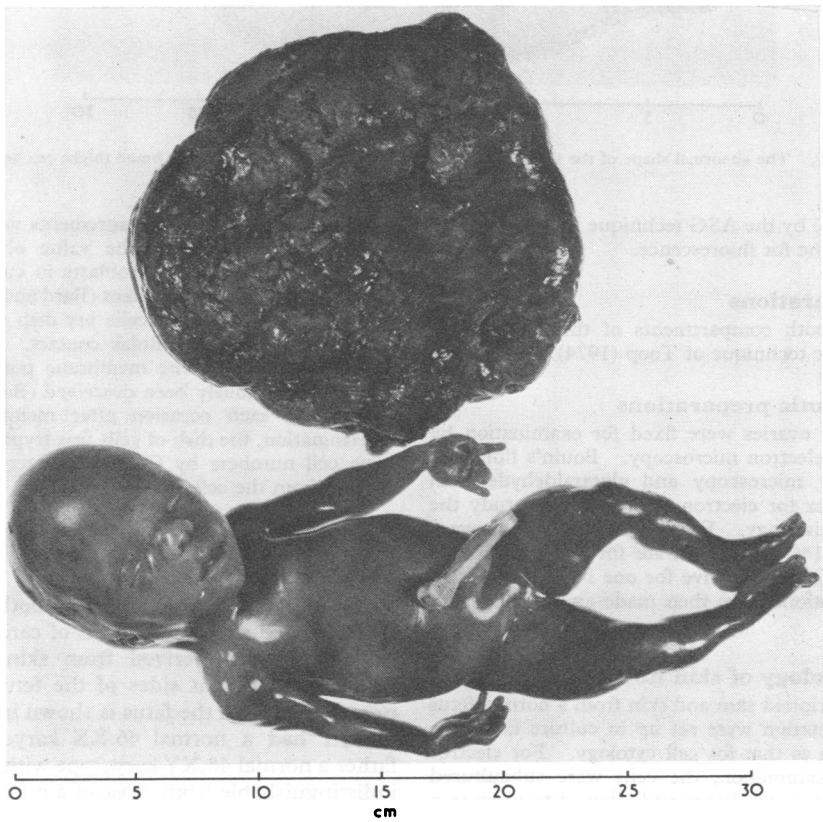

FIG. 1. Anterior view of triploid fetus and placenta. Note the dark colour of the body, syndactyly of 3rd and 4th digit of hands and feet and the wine bottle thighs. The large vesicles seen in the placenta are indicative of the hydatidiform degeneration.

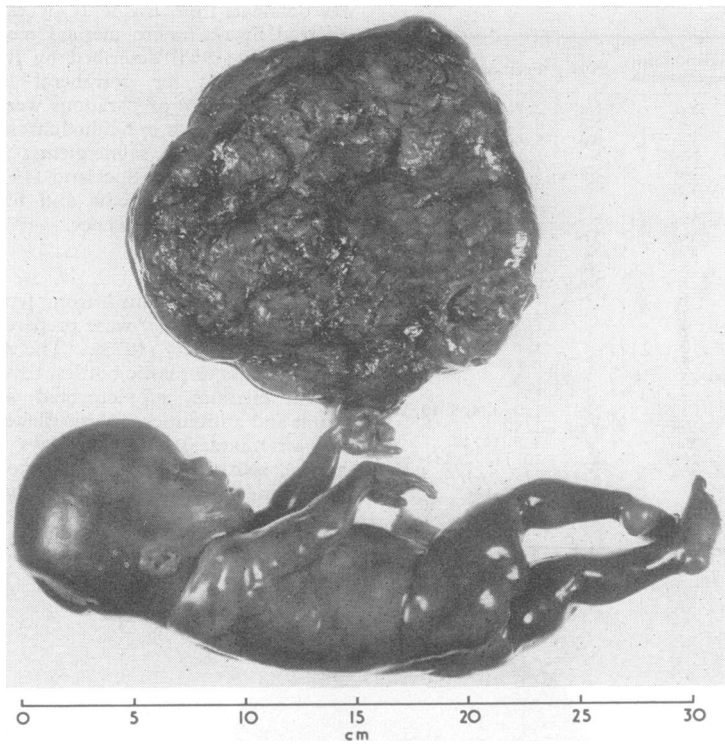

FIG. 2. The abnormal shape of the skull, low set eats, prominent forehead, and very broad thighs can be seen.

(a) by orcein, (b) by the ASG technique for banding (c), and by quinacrine for fluorescence.

Muscle preparations

Muscles in both compartments of the thighs were examined by the technique of Toop (1974).

Ovarian meiotic preparations

Pieces of the ovaries were fixed for examination by both light and electron microscopy. Bouin's fluid was used for light microscopy and glutaraldehyde with cacodylate buffer for electron microscopy to study the pathology and histology. For meiotic studies, one ovary was placed in 1% sodium citrate for 30 minutes and transferred to Carnoy fixative for one hour. Air dried meiotic preparations were then made and stained with giemsa.

Electrophysiology of skin fibroblasts

Explants of triploid skin and skin from a normal fetus of the same gestation were set up in culture using the same procedure as that for cell cytology. For electrophysiological examination, the cells were subcultured onto 5 cm plastic petri dishes and allowed to grow to a density of approximately 5.0×10^5 cells per dish, when their membrane potentials were measured. Care was taken to ensure that all measurements were made at the same cell density, since the value obtained for the membrane potential of fibroblasts in culture has been shown to be density dependent (Bard and Wright, 1974). At the density of 5×10^5 cells per dish there is a significant degree of intercellular contact. The technique for measurement of the membrane potential of fibroblasts has previously been described (Bard and Wright, 1974). On each occasion after membrane potential determination, the dish of cells was trypsinized to determine cell numbers by Coulter counter and mean cell volume from the cell diameters.

Results

Cytogenetic report

A 69,XXX karotype was found both in orcein and ASG banded cell preparations of cardiac blood and from fibroblasts derived from skin and muscles from left and right sides of the fetus. A banded preparation from the fetus is shown in Fig. 5. The mother had a normal 46,XX karyotype and the father a normal 46,XY karyotype with chromosomes indistinguishable from those of a normal female and male respectively. These results were obtained in both orcein and ASG preparations. It was hoped

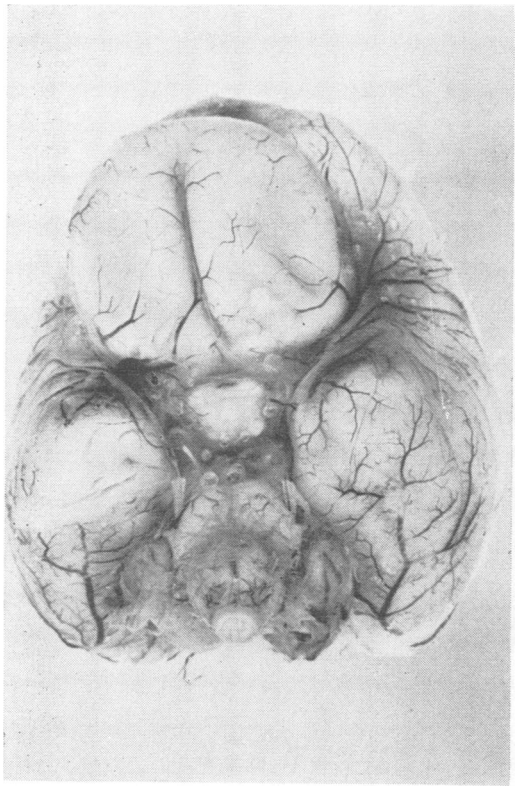

FIG. 3. The undersurface of the cerebral hemispheres showing the accessory lobe.

to show the origin of the extra fetal set of chromosomes by examining heteromorphisms present in the chromosomes of both parents and the fetus using quinacrine fluorescence. This technique requires extremely well-spread metaphases from the triploid fetus in order to reduce the intense fluorescence to a level where the detail is not obscured. It has not yet proved possible to obtain suitable preparations.

Examination of thigh musculature

In an attempt to try to explain the appearance of the thigh musculature, muscles in both compartments of the thigh were examined. On sectioning, the percentage of myotubes was higher than average for the period of gestation, showing that greater fusion and differentiation of the cells had taken place. This increased number of myotubes may in part be responsible for the well-developed appearance of the thigh musculature. There were no abnormalities either in the motor innervation or muscle histochemistry.

Ovaries and meiotic studies

Meiotic cells in leptotene, zygotene, pachytene, and diplotene were seen, and in order to obtain a quantitative estimate of the relative proportions of each germ cell stage, 100 prophase cells were counted and classified. For comparison, 100 prophase cells were counted from the ovaries of two diploid spontaneous abortuses of 26 weeks' gestation, which were chromosomally normal. The distribution of meiotic prophase stages in the triploid (73/160) and in the two diploid (73/397 73/351) fetuses is given in Table II. The distribution of cells in the triploid ovary did not differ significantly from that seen in the two diploids.

A typical pachytene oocyte from the triploid fetus is shown in Fig. 6, and for comparison a diploid pachytene öocyte is shown in Fig. 7. While all

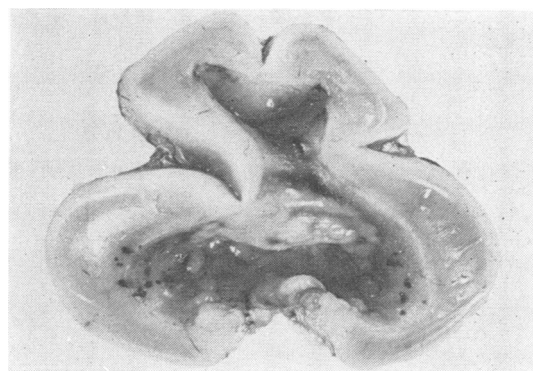

FIG. 4. A section through the posterior part of the cerebral hemispheres to show the nodules and subependymal petechial haemorrhages in the walls of the lateral ventricles.

TABLE II

DISTRIBUTION OF MEIOTIC PROPHASE STAGES IN OVARY OF TRIPLOID ABORTUS AND OF 2 DIPLOID ABORTUSES OF SIMILAR AGE

Fetus No.	Karyotype	Age of Abortion	Leptotene	Zygotene	Pachytene	Diplotene
73/160	69,XXX	25/52	6	15	17	62
73/351	46,XX	26/52	7	29	46	18
73/397	46,XX	26/52	5	15	40	40

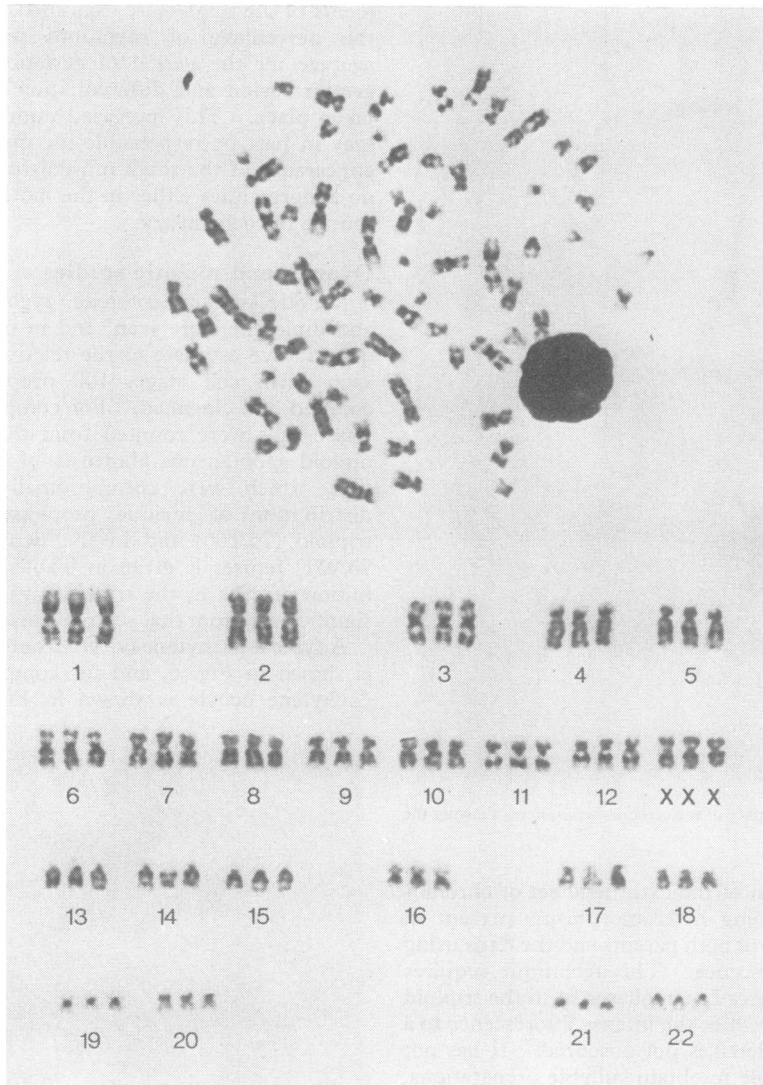

FIG. 5. A banded chromosome preparation from the triploid fetus.

TABLE III

RESULTS FOR CELL VOLUME AND MEMBRANE POTENTIAL FROM NORMAL AND TRIPLOID SKIN FIBROBLASTS

Population	Cell volume × 10^9 ml			Membrane Potential mV		
	No.	Mean	SD	No.	Mean	SD
46,XX female	75	3.28	1.41	104	10.4	2.66
69,XXX female	37	4.33	2.29	160	11.8	2.42

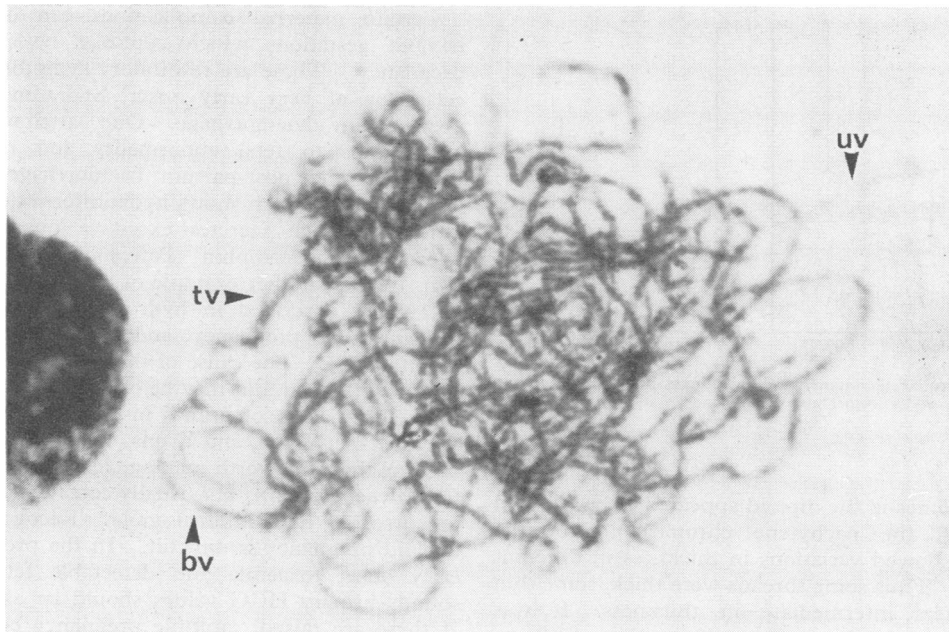

FIG. 6. Pachytene öocyte from the triploid fetus, uv = univalent; bv = bivalent; tv = trivalent.

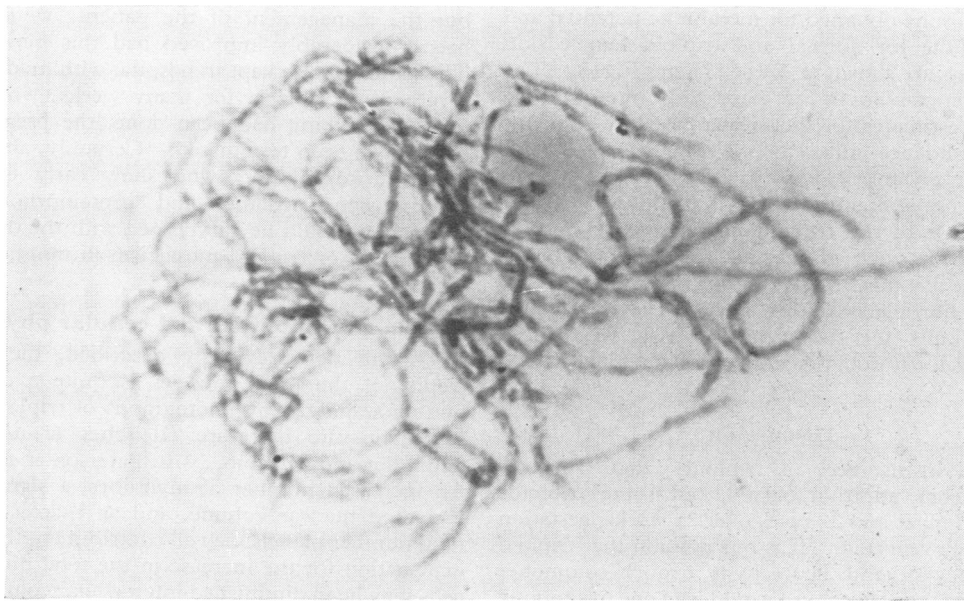

FIG. 7. Pachytene öccyte from a diploid tetus.

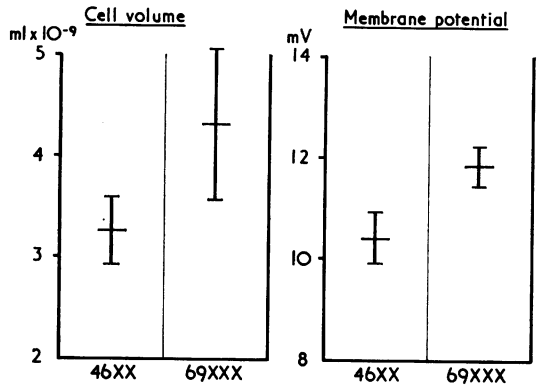

FIG. 8. Mean cell volumes and membrane potentials from the triploid fetus and a normal fetus of the same gestation.

chromosomes in the diploid appeared to be of equal thickness, the pachytene chromosomes of the triploid showed variations in thickness in different regions. Thus some threads were thick, some thin and others intermediate in thickness. It was thought that these regions of varying thickness represented univalent, bivalent, and trivalent configurations.

Electrophysiology of cultured cells

The results obtained for membrane potential and cell volume for normal and triploid female skin fibroblasts are shown in Table III and Fig. 8. The group comparisons in Fig. 8 are made in terms of the interval estimates for the means based on standard 95% confidence limits.

At the given cell density (i.e. 5×10^5 cells/dish) the mean membrane potential is significantly higher in the case of the triploid fibroblasts when compared with that of normal female fibroblasts. Though the mean cell volume is higher for the triploid fibroblasts at this density, the 95% confidence limits for the means overlap with those obtained for fibroblasts from a normal female.

Discussion

In most instances of triploidy, first trimester spontaneous abortion occurs, but some triploids survive longer and 22 cases over 24 weeks, gestation have been reported. It is our opinion that triploid pregnancy beyond 24 weeks is a much commoner condition than this figure would lead one to suppose. We have had 3 cases in approximately 7500 deliveries.

Obstetric complications

Certain maternal complications can occur in triploid gestations which continue beyond mid pregnancy. These are respiratory symptoms, pre-eclampsia of very early onset, hydramnois, and post-partum haemorrhage. One attributes the hydramnois to fetal abnormality and the pre-eclampsia and post-partum haemorrhage to the large placenta which shows hydatidiform degeneration and which secretes excessive quantities of choronic gonadotrophin (Paterson *et al*, 1971). This then is another example of 'hyperplacentosis', as already described in hydrops fetalis, diabetes mellitus, twin pregnancy, and hydatidiform mole (Scott, 1958). The cause of the respiratory symptoms is unknown, but may be of the same nature as those that occur sometimes in hydatidiform mole (Hendrickse, Willis, and Evans, 1965; Page, 1972). We feel that it is worth making clear that hydatidiform degeneration is not usually considered to be a precursor of hydatidiform mole. Recognition of triploid pregnancy is difficult. In the presence of early onset toxaemia and detectable fetal heart sounds urinary HCG values should be estimated. If these are raised, multiple pregnancy coexistent hydatidiform mole, or triploidy should be suspected. Ultrasonic scan should enable the first two diagnoses to be excluded, the latter could be confirmed by diagnostic amniocentesis. We have seen only two cases in three years where this would be indicated, but the management of the patients would have been considerably improved had this been done. The patients were kept in hospital with moderate to severe pre-eclampsia for many weeks, whereas if fetal karyotyping had been done, the pregnancies could have been terminated. Certainly, all fetuses delivered to mothers who show early onset of hypertension, oedema, and proteinuria during pregnancy should be karyotyped with the diagnosis of triploidy or triploid mosaicism in mind.

Muscle abnormalities and cellular physiology

Though not previously described, the hypertrophy of the thigh muscles is quite a common finding as judged by photographs of triploids presented in the literature (Beischer *et al*, 1967; Schindler and Mikamo, 1970; Paterson *et al*, 1971). An increased number of myotubes, a significantly increased nuclear volume, and an increase in the total number of cells may all contribute to this. An explanation for the increase in the total number of cells may lie in diminished intercellular communication. This could also occur in the heart, accounting for the cardiomegaly.

Membrane potentials in the triploid skin fibroblasts were significantly higher than those of normal fibroblasts from a fetus of comparable gestation, even allowing for altered cell volume and density in triploid cells. In view of this, one of us (MW) is studying membrane resistance, cell coupling, and sodium pump sites in triploid cells.

Meiotic studies

Though there is no report of meiotic studies in a mammalian triploid, the mode of association of the three chromosomes in triploidy is known in several plant species (McClintock, 1929; Satina and Blakeslee, 1937a, b; Moens, 1969). All 3 chromosomes may associate together as a trivalent, but synapsis at any one point never occurs between more than 2 of the 3. Some or all of each chromosome is always left unpaired. Thus in the triploid individual trivalents, bivalents, and univalents may be found in the same nucleus.

Central nervous system abnormalities

The central nervous system of the triploid fetus has often been shown to be abnormal (Table I) and in our case the orbital surfaces of the frontal lobes (part of the limbic system) were abnormal. The limbic system is intimately interrelated with the hypothalamus which controls the adrenal cortex through the anterior pituitary. Lack of stimulation of the hypothalamus by the abnormal limbic system could explain the hypoplasia of the fetal adrenals and the low maternal urinary oestriols.

In a survey of 11 000 newborn infants (S. G. Ratcliffe, 1974, personal communication) there was no association of chromosomal abnormality with major central nervous system malformations. Every case with a central nervous system abnormality was chromosomally normal. It is thus astonishing to find that 25% of late triploids have meningomyelocele and hydrocephalus, and a significant proportion of the rest have ventricular defects, both of lateral and hindbrain ventricles. This then is the only known association of a chromosomal defect with meningomyelocele and hydrocephalus. It has been suggested as a result of monozygotic twin studies where there is a discordance for spina bifida and anencephaly that these conditions are not entirely genetically determined. Carter (1965) has suggested that they depend on a polygenic predisposition interacting with intrauterine disturbances of a relatively minor kind.

This explanation of gene disturbance may account for the presence of meningomyelocele in triploidy.

Chromosomal imbalance and biological variability

A wide variety of malformations has been reported in triploids. The odd feature is that while in some triploids a particular defect may be severe, in others the defect is not present. When triploids are compared with a trisomy, e.g. Down's syndrome (trisomy 21), Edward's syndrome (trisomy 18), Patau's syndrome (trisomy 13), the pattern of abnormality in each trisomy is more constant. This is not surprising since though the mechanism by which the triploid genotype exerts its effect upon the phenotype is not known, it must involve a different interaction of regulatory genes upon structural genes, from that in the normal diploid individual. This effect can be seen clearly in the behaviour of the sex chromatin. In the case of X chromosome inactivation in triploids, the number of sex chromatin masses varies with individuals—the 69,XXX females sometimes have one inactive X chromosome, or sometimes two. Similarly 69,XXY males have either one or none. It has been postulated that there is autosomal control of X inactivation (Hamerton, 1971). Human diploid cells have one active X and tetraploid cells two active X chromosomes. Triploids thus have a complex problem of balance. Are only three sets of autosomes sufficient to inactivate two X chromosomes, or do triploid cells require two active X chromosomes?

Conclusions

The ways in which a triploid zygote may be formed are: (1) failure of the first maturation division in the mother so that the first polar body is not shed; (2) failure of the second maturation division in the mother so that the second polar body is not shed (both these processes give rise to a diploid ovum); (3) first or second meiotic non-disjunction in the father, giving rise to diploid sperm; or (4) dispermy. There is no evidence in Table I to implicate oral contraception in the origin of triploidy, nor is there sufficient detail in reported cases to assess the role of late conception. The technique being used to study the origin of the extra haploid set of chromosomes in triploids will help to shed light on this problem.

Of all conceptions 1% is triploid. Nearly all abort early, but a number, greater than is generally realized, progress beyond mid-pregnancy and sometimes cause serious obstetric complications. It is, therefore, a very significant problem in obstetrics and a fascinating natural experiment in genetics, unexpectedly throwing light on the aetiology of spina bifida.

We would like to thank Dr Ann Chandley for meiotic studies, Dr J. Moloney for the neuroanatomical sections, Dr J. Toop for investigation of motor innervation, Mrs J. Fletcher for technical assistance, and Mr N. Davidson for photography. We are particularly indebted to Professor H. J. Evans for his helpful discussions.

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