CHROMOSOMES IN PAEDIATRICS

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THE study of chromosomal morphology in relation to human disease dates from the discovery by Barr, Bertram and Lindsay (1950), of an intranuclear chromatin mass in cells of females. Such cells are termed 'chromatin positive'. Though the initial histological observations were made in laboratory animals they were soon found to be true for man (Moore, Graham and Barr, 1953). Normal human females are chromatin positive, and normal males chromatin negative. Later the investigation of nuclear sex was greatly simplified by the examination of cells smeared from the buccal mucosa (Moore and Barr, 1955). It was subsequently discovered that males with Klinefelter's syndrome are chromatin positive (Plunkett and Barr, 1956) and females with Turner's syndrome chromatin negative (Wilkins, Grumbach and van Wyk, 1954).

The next major advance was the detailed study of chromosomes by the squash technique. The normal number of chromosomes in human cells, previously thought to be 48, was then found to be 46 (Tjio and Levan, 1956). The chromatin body of Barr was found to correspond with the XX pair of sex chromosomes in the female and it was established that in chromatin-positive Klinefelter's syndrome the sex chromosomes consist of XXY (Jacobs and Strong, 1959), and that in chromatin-negative females with Turner's syndrome there is a single X and no Y (Ford, Jones, Polani, de Almeida and Briggs, 1959). Abnormalities have been detected in the non-sex chromosomes (autosomes) as well as in the sex chromosomes; thus Lejeune, Gautier and Turpin (1959) and Jacobs, Baikie, Brown and Strong (1959) showed that in mongolism there is an extra autosome, so that instead of a pair of chromosomes 21, there are three (trisomy). These techniques have much to offer in the study of disease in childhood and it is our intention to summarise the present state of knowledge of

chromosomal abnormalities with particular reference to paediatrics.

Methods and Findings in the Normal

In Barr's method epithelial cells in a smear taken from the buccal surface of the cheek are examined. The process is painless, simple and reliable and is easily performed even in infants. The best results are obtained if the smears are taken and stained by someone experienced in making such preparations but if necessary the fixed smears can be posted to the laboratory for staining and examination. If only well-stained cells are examined, Barr bodies are seen in about 40% of female cells and not in male cells. The method can be applied to fixed tissues obtained at biopsy or autopsy, and by ante-natal examination of amniotic fluid the foetal sex can be determined (Riis and Fuchs, 1960). 'Drumsticks' in polymorphonuclear leucocytes probably correspond to the chromatin bodies of tissue cells. In females they are present in less than 10% of leucocytes. Male leucocytes contain smaller, similar bodies but no true 'drumsticks'. The relatively low positive count in normal females and the possible confusion between true and pseudo 'drumsticks' make this a fallible examination in inexperienced hands, but those who practice it regularly have proved its reliability (Davidson and Smith, 1954; Davidson, 1960). Details of these techniques and their application are given by Barr (1960).

Buccal smears are of no value in the study of autosomes, but precise information concerning all chromosomes can be obtained by culture methods. There are two techniques in general use. These are the short-term culture of leucocytes from 10 to 20 ml. of blood (Hungerford, Donnelly, Nowell and Beck, 1959) or bone marrow (Ford, Jacobs and Lajtha, 1958), and the long-term culture of skin or muscle. The first involves the incubation of the material in a growth medium

FIG. 1.—The chromosomes of a normal leucocyte.

until the number of mitotic (somatic cell) divisions reaches a maximum: colchicine is then added to inhibit the formation of the cell spindle and mitosis is arrested in metaphase when the chromosomes are most clearly defined. The cell suspension is treated with hypotonic sodium citrate which swells the cells and aids the spreading of chromosomes. After fixation the cells are stained, usually by the Feulgen technique. The longer-term culture of skin or muscle requires a more elaborate technique, involving tissue culture methods (Harnden, 1960), which is probably out of the range of most hospital laboratories.

The normal chromosomal pattern is shown in

Fig. 1. In Fig. 2 the chromosomes are numbered according to the Denver (1960) Convention. The abnormalities of sex chromosomes and autosomes so far reported are given in Tables 1 and 2 respectively. These abnormalities are the result of one of two processes, non-disjunction and translocation.

Mechanisms of Non-Disjunction and Translocation

In *non-disjunction* a pair of chromosomes fail to separate during cell division and both pass into one of the daughter cells. If this occurs during meiosis the resultant gametes will show chro-

FIG. 2.—Idiogram of normal chromosomes numbered according to the Denver Convention (1960). Only one of each pair of autosomes represented.

FIG. 3.—Non-disjunction in gametogenesis and the consequences of fertilization by normal and abnormal gametes.

		Gametes			
		Normal		Non Disjunction	
Normal	♀	X	X	XX	O
	♂	X	XX	XX	XXX
	Y	XY	XY	XXY	YO
	XY	XXY	XXY	XXXX	XY
	O	XO	XO	XX	OO
Non Disjunction		X	X	XX	O
XX		XX	XX	XXX	XO
XY		XY	XY	XXY	YO
XXX		XXY	XXY	XXXX	XY
XXY		XXY	XXY	XXXX	XY
XXXX		XXXX	XXXX	XXXX	XY
XO		XO	XO	XX	OO
YO		YO	YO	XX	OO
OO		OO	OO	XX	OO

XX = Normal Female

XY = Normal Male

XXX = Superfemale

XXY = Klinefelter

XXXX = Primary Amentia

XO = Turner

YO = Non Viable

OO = Ditto

TABLE 1.—CYTOLOGICAL FINDINGS IN HUMAN SEX ABNORMALITIES

Clinical Condition	Pheno- type	Sex Chromatin	Sex Chromosome Constitution	Reference
Klinefelter's syndrome . .	Male	Positive	XXY	Jacobs and Strong (1959).
			XXYY	Muldal and Ockey (1960).
			XXY/XX	Ford, Polani, Briggs and Bishop (1959). Hayward (1960).
Turner's syndrome . .	Female	Negative	XO	Ford, Jones, Polani, de Almeida and Briggs (1959). Fracarro, Kaijser and Lindsten (1959).
		Positive	XX/XO	Ford (1960a) and (1960b).
? Turner's syndrome . .	Female	Positive	XXX/XO	Jacobs, Harnden, Brown, Goldstein, Close, MacGregor, Maclean and Strong (1960).
Super female	Female	Positive	XXX	Jacobs and others (1960). Fraser, Campbell, MacGillivray, Boyd and Lennox (1960).
Testicular feminization . .	Female	Negative	XY	Jacobs, Baikie, Brown, Forrest, Roy, Stewart and Lennox (1959). Puck, Robinson and Tjio (1960).
Primary amentia . .	Male	Positive	XXXY	Ferguson-Smith, Johnston and Handmaker (1960).
True hermaphroditism . .	Male ?	Positive	XX	Harnden and Armstrong (1959). Hungerford and others (1959). De Assis, Epps and Bottura (1960).
			XX/XXX/XO	Ferguson-Smith, Johnston and Weinberg (1960).
	?	Negative	XO/XY	Hirschhorn, Decker and Cooper (1960).
Intersex	Female ?	Negative	XO/XY	Blank, Bishop and Caley (1960).

mosome unbalance, some containing two of the particular chromosome, and others none. The possible results of non-disjunction of the sex chromosomes in meiosis, i.e. during gametogenesis, and the consequences of fertilization by normal and abnormal gametes are shown in Fig. 3. It is noteworthy that very rarely normal zygotes may result from non-disjunction in both parents, both sex chromosomes of the female zygote being derived from the mother, and both those of the male from the father. Non-disjunction may also effect autosomes and will result in autosomal trisomy or monosomy. Non-disjunction during mitosis after formation of the zygote results in a cell population showing more than one chromosomal pattern (mosaicism). The type of mosaicism depends upon the stage of development of the zygote at the time of non-disjunction (Fig. 4). When it occurs at the first cleavage division the result will be two-cell types, but when it occurs at the second or a subsequent division

three-cell types will be produced. In Turner's syndrome for example, we have recently observed a mosaic of three-cell types (XXX/XX/XO).
Translocation involves reciprocal transfer of segments of chromosomes by a process of breakage and re-union between non-homologous chromosomes (Fig. 5). When unequal segments are involved translocation can be detected at mitosis since the chromosomes cannot be paired off as in the normal idiogram. When equal segments are involved it can only be detected during meiosis in gonadal tissue when an unusual configuration of four chromosomes will be seen. Translocation does not affect the individual in whom it occurs as the total chromosomal content is unaltered, but depending upon the behaviour at meiosis an abnormal gamete may result. Fertilization by a normal gamete will result in the production of a zygote with an unbalanced chromosome content. An example of this has been described in a child with 'polydyspondylie' (Turpin, Lejeune,

TABLE 2
HUMAN AUTOSOME ABNORMALITIES

Clinical Condition	Autosome Abnormality	Reference
Mongolism	Trisomy chromosome, 21	Lejeune and others (1959). Jacobs, Baikie, Brown and Strong (1959).
	Translocation anomaly	Polani and others (1960).
Peculiar facies and digits Webbed neck Ventricular septal defect of heart	Trisomy chromosome, 17	Edwards and others (1960).
Cerebral defect Simian creases Trigger thumbs Polydactyly Capillary hæmangiomata Hare lip Cleft palate Apparent anophthalmia Heart defect	Trisomy chromosome, 15	Patau and others (1960).
Sturge-Weber syndrome	Trisomy chromosome, 22	Hayward and Bower (1960).
Normal (father of a mongol)	Trisomy chromosome, 19	Fracarro and others (1960).
Polydyspondylie	Translocation anomaly	Turpin and others (1959).

Lafourcade and Gautier, 1959), in whom the greater part of one of the smallest chromosomes was found translocated on to one of a longer pair.

Selection of Patients

As full chromosomal examination is so time-consuming it can economically be applied only to a small proportion of patients. Careful selection of those to be studied is essential and will depend on research as well as diagnostic considerations.

Virilization in females, ranging from simple enlargement of the clitoris to more complete pseudo-hermaphroditism, may make diagnosis of sex difficult. In these patients the buccal smear is chromatin-positive and full chromosomal investigation is not essential. Most of these cases are instances of the adreno-genital syndrome, and confirmation of the diagnosis can be rapidly obtained by the pattern of urinary steroid excretion (Hill, 1960). Others may be due to androgenic substances, including progestational steroids, given

to the mother during pregnancy, and occasionally no cause can be found.

The great majority of patients with hypospadias and apparently undescended testes are in fact males, nevertheless male sex should be confirmed by a buccal smear (chromatin-negative) before surgical correction is undertaken. In the syndrome of testicular feminization the external genitalia are typically female. The testes may be intra-abdominal, in the labia majora, or in the inguinal canals, where they may be mistaken for herniæ or enlarged glands. The buccal smear is chromatin-negative and confirmation of male sex may be obtained by the finding of an XY sex chromosomal constitution (Jacobs, Baikie, Brown, Forrest, Roy, Stewart and Lennox, 1959).

True hermaphroditism with male and female gonadal elements is rare. The external genitalia show male and female features. In the majority the buccal smear is positive (Barr, 1955), and the chromosomal constitution XX (Ferguson-Smith, Johnston and Weinberg, 1960) but a case with mosaicism (XO/XY) has been reported recently (Hirschhorn, Decker and Cooper, 1960). The diagnosis rests upon biopsy of the gonads. Forster (1960a) has suggested that there may be gonadal mosaicism and the chromosomal constitution of the gonads and somatic tissues should be fully investigated.

These various forms of intersex can present difficult diagnostic problems in infancy or early childhood. It is a matter of urgency to establish the proper sex as soon as possible after birth (Moore and Edwards, 1960; Wilkins, 1957). In reaching this decision determination of the chromosomal sex is essential, though it is by no means the only factor to be considered (Money, Hampson and Hampson, 1956).

In the conditions discussed above chromosomal studies are used to establish the proper sex of the patient and the sex chromosomes correspond to the gonads. True abnormalities of the sex chromosomes are more likely to manifest themselves at puberty since they result in deviations from the normal development of secondary sexual characteristics. Two such conditions, Turner's syndrome and Klinefelter's syndrome, are now well defined. In both there may also be mental retardation so that examination of the buccal smear in retarded children may lead to the diagnosis before puberty (Ferguson-Smith, 1958). The diagnosis of Klinefelter's syndrome is otherwise unlikely to be made during childhood though it might be suggested by the finding of unusually long legs (Tanner, Prader, Habich and Ferguson-Smith, 1959). In Turner's syndrome mental retardation occurs in only a minority, but the other characteristic abnormalities often enable

FIG. 4.—The types of mosaicism resulting from nondisjunction in the first and subsequent cleavage divisions. The shaded segments are presumed to be non-viable.

Equal translocation. Non identifiable in mitotic cells.

Unequal translocation. Identifiable in mitotic cells.

FIG. 5.—Translocation.

the diagnosis to be made during childhood. Ford (1960b) has found mosaicism (XX/XO) in a high proportion of cases of Turner's syndrome. In our patient with triple mosaicism ($XXX/XX/XO$) already referred to, an abnormal sex chromosome

constitution was suggested by a low percentage of chromatin-positive cells in the buccal smear and by the finding of double Barr bodies.

Autosomal abnormalities should be suspected in patients with multiple congenital defects in whom the buccal smear is normal. The chief example of this group is mongolism. This condition is usually the result of trisomy for chromosome 21 but Polani, Briggs, Ford, Clarke and Berg (1960) have described a mongol with an abnormality of one of pair 15. There was no trisomy in the usual sense but they postulate that the abnormality was the result of translocation in a previous generation of the greater part of a chromosome 21 on to one of pair 15. The mother of this child was only 21 years old and the authors suggest that this or a similar mechanism, rather than the usual trisomy, may be involved in mongols of young mothers.

Another infant with multiple congenital abnormalities was found to be trisomic for chromosome 17 (Edwards, Harnden, Cameron, Crosse and Wolff, 1960), and a somewhat similar patient found to be trisomic for chromosome 18 was described by Patau, Smith, Therman, Inhorn and Wagner (1960). Undoubtedly other examples of autosomal trisomy will be discovered, though when the larger chromosomes are involved the

condition is likely to be incompatible with embryonic survival. In general, autosomal trisomy should be suspected in patients with multiple structural abnormalities rather than in patients with single localized deformities (Edwards *et al.*, 1960). Single gene defects are not associated with any morphological chromosomal abnormality detectable by present methods.

Research Aspects

Important advances are to be expected in the field of chromosomal research. Some of these will stem from the investigation of clinical problems as outlined above, and others from the systematic investigation of specific conditions. Further studies in leukaemia, where abnormalities have already been found (Ford, Jacobs and Lajtha, 1958; Baikie, Brown, Jacobs and Milne, 1959; Nowell and Hungerford, 1960) are likely to be rewarding. Willis (1958) stresses the need for more detailed investigations into the nuclear sex of teratomas. Much attention has been paid to the chromosomes in malignant tumours; the study of embryonal tumours should be of particular interest. Because certain chromosomal abnormalities are likely to be incompatible with life, early abortions should be examined. Clinical conditions occurring predominantly in one sex are worthy of attention as abnormalities of sex chromosomes may occasionally be brought to light. For instance we have recently encountered a girl with Hirschsprung's disease, which occurs predominantly in male children, in whom mosaicism was suggested by the buccal smear, which

showed only 10% chromatin positive cells, and confirmed by examination of the chromosomes (XO, XX, XXX). Before attaching much significance to such findings other cases must be examined to exclude the possibility of chance association. For example, since one of us (Hayward and Bower, 1960) reported a case of Sturge-Weber syndrome with trisomy for chromosome 22, we have examined seven other cases and found no chromosomal abnormality (Hayward and Bower, 1961). So far, chromosomal abnormalities in Klinefelter's syndrome, Turner's syndrome and mongolism are the only ones in which repeated observations have adequately confirmed the initial findings. Apart from establishing consistent chromosomal abnormalities in specific conditions future research must elucidate the mechanisms whereby variations in the chromosomal pattern lead to distinctive clinical syndromes. In any such investigation a greater knowledge of the range of variation of chromosomal pattern in normal individuals is required. Already a clinically normal person has been found with trisomy for chromosome 19 (Fracarro, Kaijser and Lindsten, 1960) and indeed Kodani (1958) claims to have observed such supernumerary chromosomes frequently in male meiotic material. Confirmation of these results however, is hampered by the shortage of suitable testicular material.

In conclusion, we wish to stress the need for close co-operation between cytologist and clinician so that the limited laboratory facilities available may be used to the best advantage.

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