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Human Cytogenetics

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Introduction

May I begin by recalling that at the Copenhagen Conference five years ago, Tjio and Levan had just published their almost apologetic announcement that they could only find 46 chromosomes in cultured human somatic cells, and Hamerton and I presented evidence that there were 23 bivalents in spermatocytes, not 24.

Since that time there has been a very marked expansion of work in human cytogenetics. The reason is not hard to seek: the findings have been of significance in so many fields of medicine ranging from endocrinology, through paediatrics, gynaecology and mental diseases to haematology and cancer.

To the biologist, this outpouring of interest may seem at times to have bordered on the hysterical. For him the developments may have done little more than illustrate in our own species the principles that were established in the twenties and thirties with other material. On second throughts he will realize that man offers unique advantages for cytogenetic study in virtue of the enormous numbers of individuals who are subjected to medical scrutiny and thereby become candidates for chromosomal examination. But he will also remember the serious disadvantages, (some shared with human Mendelian genetics) of long generation time, small family size, impossibility of experimental mating and lack of opportunity (except perhaps rarely in males) to study the meiotic pairing which can be so revealing of homology.

If opportunities for direct test of an interpretation are few, reliance on general hypotheses must be the greater. I make it a rule to doubt an observation if it is not in accord with hypothesis. This is not to reject Bateson's exhortation to " treasure your exceptions "; but one must first make quite sure that they *are* exceptions. Perhaps one does not make so many new discoveries this way, but fewer may later be shown not to be discoveries after all.

Sex Chromosome Abnormalities

I do not propose to say anything about technical matters here other than to remind you that there are three general methods, namely, short-term marrow culture, culture of peripheral blood, and true tissue culture, and that all are designed to provide dividing cells *in vitro* on which the technician may exercise his arts.

I shall avoid the term " mongolism ". Reasons for preferring another were given in a letter with 19 signatories that appeared in the Lancet earlier this year. I shall speak of the " Down syndrome ".

https://doi.org/10.1017/S1120962300017984 Published online by Cambridge University Press

Historically, the extra small chromosome in cases of the Down syndrome was the first abnormality to be discovered — by Lejeune, Turpin and Gautier in Paris and published in January 1959. Since then, as all know, many others have been found.

Seven types of simple numerical abnormality of the sex chromosomes are now established. Wherever a Y chromosome is included the patient bears testes and is clinically recognisable as a case of Klinefelter's syndrome. All the types that lack a Y are females. The XO cases exhibit Turner's syndrome; the XXX and XXXX cases are clinically unremarkable, but may be subnormal mentally.

The identification of so many types would not have proceeded so rapidly were it not for the invaluable aid in detection given by buccal smear examinations for sex chromatin. There is much that could be said about the nature and significance of sex chromatin but time does not permit me to do more than mention the important empirical rule that the maximum number of sex chromatin bodies is one less than the number of presumptive X chromosomes present.

Some females have been found to contain a single abnormal chromosome in an otherwise normal set of 46. There is good reason to believe that these abnormal chromosomes are derivatives of the X. They include two types of presumptive deficiency and another type, larger than normal, which is believed to represent a symmetrical duplication of the long arm about the centromere and is referred to as an iso-chromosome. Careful morphological and cytogenetic comparison of cases like these may provide us with some understanding of the action of different regions of the X chromosome, as Westergaard has done in the plant *Melandrium rubrum*.

In addition to the simple numerical irregularities a class of individuals is now recognised, whose bodies, the evidence suggests, are compounded of two or three types of cell that differ in their content of sex chromosomes. These *mosaics* presumably owe their origin to mitotic errors during early development. At least 11 different types have been recorded in the literature.

The occurrence of mosaicism makes it possible to understand a case of outstanding interest recently reported by Lejeune and his collaborators, namely apparent one-egg twins that were male (XY) and female (XO) respectively. The evidence of monozygosity includes concordance for a considerable number of red-cell antigens and acceptance of reciprocal skingra fts.

All the observations on individuals with abnormal sex chromosomes I have mentioned so far are consistent with a simple hypothesis, namely that presence of a Y chromosome determines that the promordial gonad shall become a testis. And the testes, the embryologists and endocrinologists tell us, determine the masculine differentiation of the internal and external genitalia during embryogenesis as well as masculine secondary sex development at puberty. The existence of two types of XY female does not require the rejection of this hypothesis. They merely show that the Y, though it may be *necessary* for masculine development, does not *ensure* it. The Y cannot transform the primordial gonad into a testis if there is no primordial gonad to transform, as in pure gonadal dysgenesis; and in testicular feminisation testes *are* formed, the failure of masculinisation of the external genitalia and the feminine direction of secondary sex development apparently being determined by a simple Mendelian gene.

True hermaphroditism, however, *does* provide difficulties for the simple hypothesis. By definition, both testicular and ovarian tissue are present in the affected individuals. Mosaicism,

such as in the XO/XY case of Hirschhorn and his colleagues, may be the explanation. But in at least six cases only XX cells have been identified. Even these *could* be cryptic cases of XX/XXY mosaicism though this may be pushing the idea to the limits of credulity. I do so to underline the need to study cultures established directly from gonadal tissue whenever such cases come to laparotomy.

The alternative to some form of mosaicism, genetic or chromosomal, is to follow Goldschmidt and assume that masculinizing *and* feminizing factors are present in the chromosomes of *both* male *and* female, but that normally one set decisively outbalances the other: but where the balance is equivocal, chance local factors operating during embryogenesis may tip the scales in different directions in the two gonads or even in different parts of the same gonad. If these considerations have any validity, the situation is conceivable where both gonads become testes in a chromosomally XX individual. A case recently reported by Shah and his colleagues may be an example. But we need to be sure. And to be sure means the effective exclusion of mosaicism by a cytogenetic study of as many tissues as possible, and certainly the gonads.

The abnormal males with Klinefelter's syndrome are all sterile; of the abnormal females, however, several of the XXX cases and one example of XO Turner's syndrome have proved to be fertile. The XXX cases are of special interest. One would expect them to produce XXX ova in approximately equal ratio with normal X ova, and so to be mothers of Klinefelters and more triplo-Xs. Yet of the 10 children born to XXX mothers so far recorded all are apparently normal sons or daughters. We may compare the proportion of normal children to Down syndrome children born to Down syndrome mothers. A search of the literature made by my colleague Dr. Clarke gives a ratio of 8 normals to 5 cases of Down's syndrome. Although the formal position — the segregation of a presumptive trivalent association during oocyte meiosis — is very similar, the outcome is very different. In the one situation (triplo-21) secondary nondisjunction evidently occurs frequently: in the other (triplo-X) there is, as yet, no evidence that it occurs at all.

Autosomal Abnormalities

Turning now to abnormalities of the autosomes, two types of primary autosomal syndrome with extensive congenital abnormalities have been established in addition to the Down syndrome. It is significant that in these cases also there appears to be a marked association with advanced maternal age.

The remarkable diploid/triploid mosaic boy of Böök and his colleagues no longer stands alone since triploid tissue has been identified in two aborted foetuses by Penrose's group. In one, diploid cells were also found, so providing a more direct resemblance with the Uppsala case. This work has the important additional value of demonstrating that the establishing of cultures for chromosome study from spontaneously aborted embryos is technically feasible, even when the foetus has been dead for some time and the tissues are largely macerated. Determination of the karyotypes of aborted foetuses could be particularly valuable where the mother is approaching the menopause, in instances where there is known chromosomal abnormality in either parent, and also where abortion and normal live births occur in erratic sequence.

Mosaicism involving the autosomes seems to be relatively much less frequent than mo-

saicism of the sex chromosomes: in addition to the two 2n/3n cases, whose origin may have been in errors of fertilization rather than mitosis, three examples have been reported in cases of the Down syndrome. One is a normal/triplo-21 mosaic in whom effectively normal intelligence for age is associated with a number of Down-syndrome stigmata. Three cell-lines were found in the other two examples. In both mentality was sub-normal.

The first example of a presumptive reciprocal translocation, or rather, translocation derivative (since only one of the expected two rearranged chromosomes was identified), was the Turpin-Lejeune case of polydyspondylie. Since then it has been shown that a similar type of translocation heterozygosity can account for the inherited tendency to produce children with the Down syndrome exhibited in some pedigrees. In at least two instances hereditary transmission of the abnormal chromosome through two generations has been demonstrated. The inherited tendency is usually transmitted through females rather than males. It is reasonable to look for an explanation in the different conditions of meiosis in the two sexes.

Other published cases of abnormal translocation are few. They include a remarkable family reported by Moorhead and his colleagues in which the mother and several of her children had 45 chromosomes, the children concerned all exhibiting a severe speech defect. The rearranged chromosome found in this family is very similar morphologically to several seen in the Down syndrome translocations, apparently being derived from the long arms of a long and a short acrocentric chromosome.

Neoplasia

It is now common to hear mention of " chromosomal abnormality " in connection with neoplasia. I would urge that this expression is insufficient and may be misleading.

Most progress has been made with the study of the leukaemias. In one type, chronic myeloid leukaemia, an abnormally small acrocentric chromosome characterises the disease. This example of a specific chromosomal lesion associated with a specific clinical condition is so far unique.

In acute leukaemia the nature of the abnormality, if there is one, varies from case to case Very few have been subjected to detailed analysis as yet. In one of them all the abnormal cells exhibited precisely the same karyotype. The simple inference is that they were related by descent and constituted a clone. This implies a single cell origin of the neoplastic cell-population, or at least of an important part of it. The alternative possibility that the same abnormality arose several or many times independently is difficult to sustain.

In another case Dr. Clarke has found a mixture of 7 different cell types that show karyotypic relationship to one another and conform to a simple scheme of derivation. This, we feel, is evidence of progressive, step-wise origin of the abnormalities and support for Winge's hypothesis of selective proliferation of the more " aggressive " cell-types. The need now is to sample such cases at different times during the course of the disease.

The Identification of Individual Chromosomes

The identification of individual chromosomes is still a matter of controversy. The system of nomenclature agreed upon at Denver, whatever its shortcomings, has provided us with a

framework on which we can build. I invite you to think what the position would have been today without it. We all recognise it to have been in error in defining the acrocentric chromosomes by the presence or absence, and size, of satellites. But it is in respect of the medium-sized group that it has come in for more serious criticism from Patau. The report stated that this group presented the greatest difficulty; no attempt was made to define the individual pairs in words, but a table of measurements contributed by different authors was included without comment. A casual glance at this table reveals many inconsistencies, but if a certain amount of rearranging is done it can be seen that the inconsistencies are very much less than Dr. Patau thinks. Since different culture methods and preparative techniques were used by the different observers, others might consider the figures to show a remarkable degree of concordance. If the two longest pairs, admittedly the most difficult of all, are excluded and the order of the others rearranged in a few places, the remaining 36 entries in this section of the table fit the verbal definition of these chromosomes that Dr. Clarke and I have arrived at with only 4 real exceptions. The partial karyotype and Patau karyogram shown in figs 1 and 2 illustrate these distinctions.

Dr. Clarke and I are satisfied that the three long acrocentric pairs can also be distinguished. One has a fairly prominent short arm and often bears a relatively large satellite. Another has an inconspicuous short arm and also commonly bears a satellite. The third has a conspicuous short arm and is rarely satellited.

No one is more strongly aware than I that single illustrations do not prove anything. I nevertheless assert that, in favourable preparations, the chromosomes of the 6-12-X and 13-15 groups can be separated into pairs by visual matching and that their characteristics are repeatable from cell to cell.

The stage of gathering easy fruits in human cytogenetics may now be nearly over. If so, the more accurately we can define the normal chromosomes, the better armed we shall be to proceed further and detect minor departures from the norm, whether pathological or indicative of natural ploymorphism of the chromosomes. This is certain to be a much more difficult and exacting task: it may prove to be no less rewarding.

Comment on Paper by Dr. Morishima

Dr. Morishima said that I had suggested that XO/XX mosaics arose from XO zygotes. I favoured this possibility but this is not to say that I excluded the alternative. The original mosaics were detected in bone marrow preparations. At that time I was impressed with the possibilities there were for differential proliferation of genetically distinct cell types in bone marrow — I still am. On this basis a deficient XO cell arising from normal XX cells would seem to be less likely to be perpetuated and ultimately give rise to an important component of the soma than an XX cell arising from XO cells. This may well be true for bone marrow — as we gather more information about mosaics we should get some indication whether it is or not. But I may have been wrong in applying the idea to the very early stages of embryogenesis. I therefore repeat that I certainly do not exclude the origin of XO/XX mosaics from XX zygotes. Dr. Morishima's evidence and the genetical analysis of XO mice by Russell and her colleagues in fact support this possibility rather than the other.

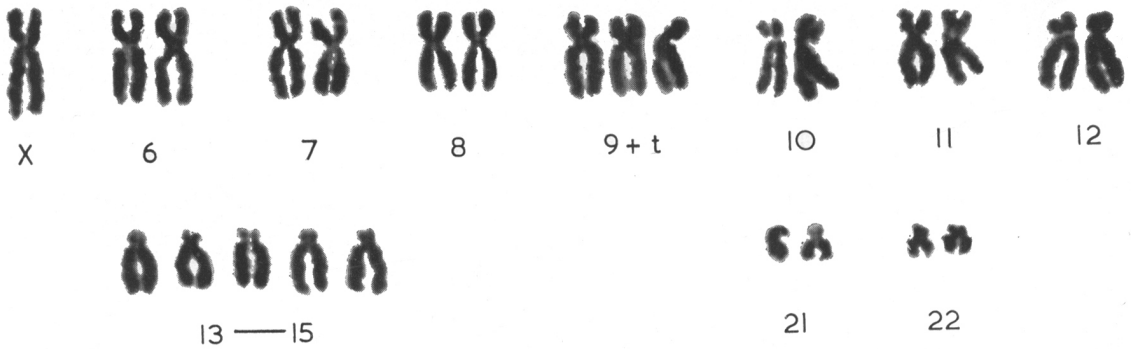

Fig. 1. Partial karyotype of a male case of Down's syndrome with 46 chromosomes. The chromosomes were paired on a visual assessment of length and centromere position, no account being taken of measurements. One chromosome is a presumptive product of a reciprocal translocation between a long acrocentric and a short acrocentric chromosome. Pairing shows that the extra chromosome (t) resembles the members of pair 9. The X chromosome is variable and in this cell has an unusually long long-arm. The chromosomes of the 13-15 group are not distinguishable in this cell

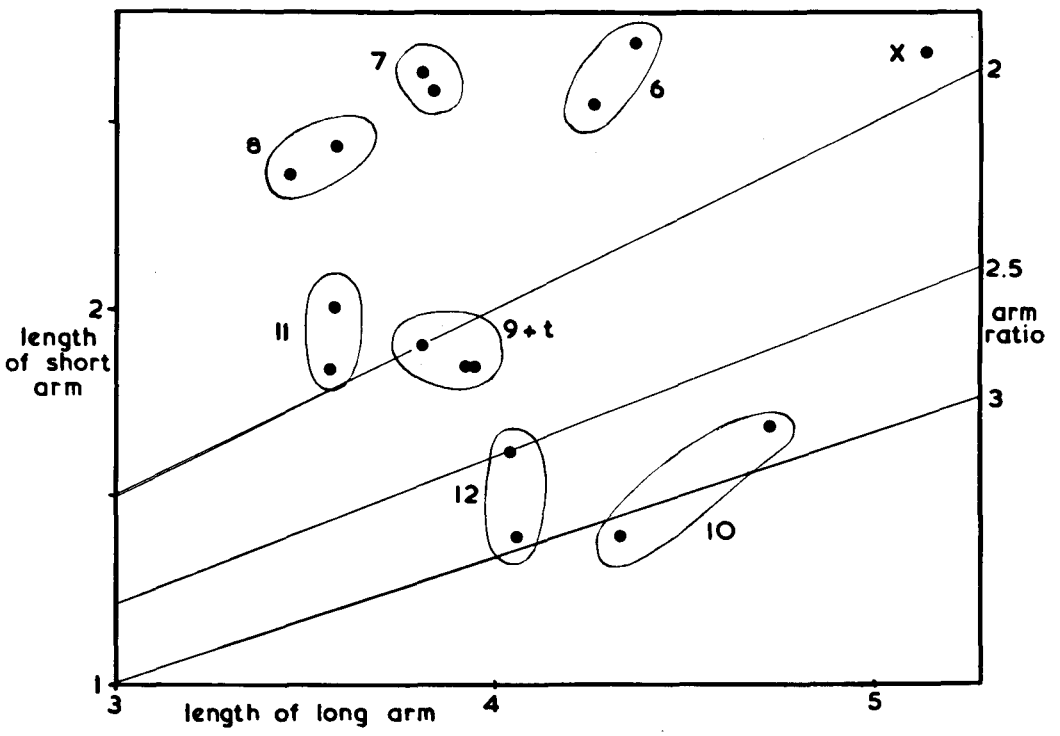

Fig. 2. Partial karyogram derived from the chromosomes of the 6-12-X group shown in Fig. 1. The measurements were made on an original enlargement before the chromosomes had been identified in pairs

https://doi.org/10.1017/S1120962300017984 Published online by Cambridge University Press

Comment on Paper by Professor Penrose

I understood Professor Penrose to say that the abnormal euploid gametes produced by reciprocal translocation heterozygotes are *either* deficient for certain chromosomal material *or* carry an excess. In fact, these gametes are simultaneously deficient and duplicated. More explicitly, each lacks a particular chromosome segment entirely and at the same time has a second segment represented twice. Zygotes would therefore be monosomic for the one segment and trisomic for the other. Whereas it is now reasonable to suppose that in our own species trisomy of a short segment might be compatible with live birth, though probably not with normality, monosomy seems likely to lead to death in utero.

Comment on Dr. Yerganian's Paper

I was impressed by Dr. Yerganian's evidence of the maintenance of the differential behaviour between the morphologically normal X chromosome and the long X chromosome derivative. The difference in time of uptake of tritiated thymidine label is evidently a permanent and consistent property. He would therefore seem to be justified in rejecting Ohno's hypothesis — for tissue cultures. It does not necessarily apply to the body. Even if it should, it would not exclude the possibility that the differential behaviour of the one X chromosome is not acquired until a relatively advanced stage of embryogenesis, with equal probability of the paternal and maternal Xs being affected in any given cell, as required by Dr. Mary Lyon's hypothesis.