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Cytogenetic studies in leucocytes on the general population: subjects of ages 65 years and more

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No results have yet been published of cytogenetic studies on a random sample of the general population. Such studies, however, are desirable for a number of reasons. First, Jacobs, Court Brown & Doll (1961), Jacobs *et al.* (1963) have reported finding an increase in the number of aneuploid cells with age in blood cultures from apparently normal individuals. These studies were made on a non-randomly selected group of individuals, and it is desirable to see whether the same effect is present in a randomly chosen group of subjects. Secondly, it is usual for small numbers of cells in blood cultures from normal individuals to contain structural abnormalities of the chromatid or chromosome type. It is known that the proportion of cells with chromosomal abnormalities can be markedly increased in blood cultures following exposure *in vivo* to high doses of radiation (Tough, Buckton, Baikie & Court Brown, 1961; Bender & Gooch, 1962, 1963; Buckton, Jacobs, Court Brown & Doll, 1962). In theory it may be anticipated that some increase will follow exposure to low doses, and the search for such an effect will be helped by knowledge of the frequency of cells with structural abnormalities in a sample of the general population.

Thirdly, it has for some time been known that, occasionally, variations occur in the morphology of the acrocentric chromosomes and chromosome no. 16, and it is of interest to determine the frequency of these phenomena (Sasaki, Makino & Kajii, 1963; Chandra & Hungerford, 1963; Chapelle, Aula & Kivalo, 1963). Finally, there have been a number of reports tending to associate apparently minor chromosomal abnormalities with pathological states (Tough *et al.* 1962; Gunz, Fitzgerald & Adams, 1962; Schmid, 1962). Some of these reported associations, however, may be fortuitous, and an important aid to determining whether this is so will be the study of the frequency of minor abnormalities and variations in the general population.

This communication reports the results of the study of 189 elderly subjects who were randomly chosen from the general population, and for whom leucocyte cultures have been examined. Particular emphasis has been laid on three kinds of investigation, the distribution of chromosome counts, the frequency of cells with chromatid and chromosome structural abnormalities, and the frequency of subjects with abnormalities or with well-marked variations of the karyotype in all their cells.

MATERIAL AND METHODS

During 1961 and 1962 the staff of the Edinburgh Geriatric Hospital Service studied the medical status and social welfare of a random sample of subjects of ages 65 years and more drawn from the lists of three general practices. One was a rural practice outside Edinburgh and two were urban practices within Edinburgh. The opportunity was taken to undertake chromosome

studies on the members of the sample. In all a sample was drawn of 110 males and of 151 females, but a number of these refused to co-operate. In the end chromosome studies were attempted on 92 males and 108 females, but in eleven instances (5 males and 6 females) chromosome preparations were not obtained, so that this report is based on the study of 87 males and 102 females for whom satisfactory preparations were obtained. The ages of these subjects and the number of cells studied are shown in Table 1.

Table 1. *Number of subjects and cells studied*

Males				Females			
Age group (years)	Mean age	No. of subjects	No. of cells	Age group (years)	Mean age	No. of subjects	No. of cells
65–69	66·9	38	1140	65–69	67·2	39	1156
70–74	72·0	25	750	70–74	71·8	25	750
75–79	77·0	11	330	75–79	76·7	21	630
80+	83·1	13	380	80+	82·2	17	510
All ages	72·1	87	2600	All ages	72·8	102	3046

Chromosome studies were made on cells cultured from the peripheral blood, using a modification of the technique of Moorhead *et al.* (1960), the final spreading of the chromosomes being achieved by drying in air. Thirty cells were counted from every individual case with the exception of two in whom respectively only twenty and sixteen cells could be counted. In each subject every cell counted was also analysed, and all cells with an aneuploid chromosome number or with a structural abnormality were checked by a second observer. Fibroblast cultures were also studied wherever possible from any subject in whom all cells in a blood culture showed a consistent abnormality or variation. Six observers were involved and the problem of observer error, particularly variations between observers in the criteria of selecting cells for counting and analysis, has to be considered in relation to the distribution of chromosome counts. A separate study was done to investigate this and also the variations between different counts done on the same individual from blood cultures set up at different times. The results of this study are the subject of an Appendix (by H. Goldstein).

RESULTS

The distributions of chromosome counts

The distributions for males and females are shown in Table 2, the counts recorded being the aggregate counts for the subjects in each of the four age groups in either sex. By comparison with the numbers of hypodiploid cells, comparatively few cells with hyperdiploid counts were found, but these show an upward trend with age. The data in Table 3 show that for males there is reasonable agreement between the proportions of hypodiploid cells found in each group and those expected to be found as calculated from the regression equation $y = 0{\cdot}082x + 2{\cdot}26$, where y is the proportion of hypodiploid cells expressed as a percentage of the diploid cells and x is the mean age in years (Jacobs *et al.* 1963). Calculations based on the equivalent equation for females, $y = 0{\cdot}161x - 0{\cdot}64$, showed the expected proportions in each instance to fall short of the observed proportions. This discrepancy is most probably due to the relationship for females being more complex than that for males, and Jacobs *et al.* (1963) felt that their data would have been better fitted by a cubic relationship, but insufficient information was available to test this.

It had been reported by Jacobs *et al.* (1963) that the increase with age in the proportion of cells

with 45 chromosomes was explained in males by an increase in cells lacking a small acrocentric chromosome, while in females the change was due to an increase in cells lacking a medium-sized chromosome. The present data, although limited in scope, bear out these conclusions. In males, and excluding cells with structurally abnormal chromosomes apart from chromatid breaks, chromatid gaps and isochromatid gaps, there were 109 cells with 45 chromosomes. In 58 of these a small acrocentric chromosome was absent, whereas on the assumption that each chromosome had the same chance of being lost, the expected number of such cells was 11·85. In cells of good quality it is possible to recognize the *Y* chromosome by its morphological features, and in 26 of the 58 cells it was evident that the *Y* chromosome was missing. The expected number, assuming each chromosome to have an equal chance of being lost, was 1·26. Among the remaining 32 cells it could be stated with certainty for only 9 that the missing small acrocentric chromosome was an autosome.

Table 2. *The distribution of chromosome counts*

	Males (no. of cells per age group)					Females (no. of cells per age group)				
Chromosome counts	65–69	70–74	75–79	80+	All ages	65–69	70–74	75–79	80+	All ages
< 44	11	3	2	5	21	7	5	5	5	22
44	13	11	2	8	34	12	3	4	8	27
45	61	32	13	26	132	91	81	66	52	290
46	1050	697	310	336	2393	1032	654	547	436	2669
47	4	4	3	5	16	13	6	7	9	35
48	—	1	—	—	1	1	1	1	—	3
> 48	1	2	—	—	3	—	—	—	—	—
Total cells	1140	750	330	380	2600	1156	750	630	510	3046

Table 3. *Comparison of the observed and calculated proportions of hypomodal cells expressed as percentages of modal cells*

	Males					Females				
	65–69	70–74	75–79	80+	All ages	65–69	70–74	75–79	80+	All ages
Observed	8·1	6·6	5·9	11·6	7·2	10·7	13·6	13·7	14·9	12·8
Calculated	7·8	8·2	8·6	9·1	8·2	10·0	10·7	11·5	12·4	10·9

Among the females, and excluding cells with structural abnormalities apart from those types already noted, there were 261 cells with 45 chromosomes. In 208 cells a medium-sized chromosome was missing, whereas, on the assumption that each chromosome had the same chance of being lost, the expected number of such cells was 90·78.

The numbers of cells with structurally abnormal chromosomes

It often happens that a few cells in a blood culture have one or more chromosomes showing a structural abnormality, sometimes together with an acentric fragment or fragments. It is known that the numbers of such cells can be markedly increased following exposure *in vivo* or *in vitro* to ionizing radiations. It also appears that a temporary increase in some form of structural abnormality may follow certain virus infections (Nichols, Levan, Hall & Ostergren, 1962; Harnden, 1963; Aula, 1963).

In studying these cells the following classification has been used, based on that of Buckton *et al.* (1962).

Type A cells

These are cells with no evidence of a structural abnormality. They are subdivided as follows:

(1) A_1 cells with an apparently normal diploid chromosome complement.

(2) A_2 cells with no apparent structural abnormalities, but with either a hypodiploid or hyperdiploid chromosome number.

Type B cells

These are cells in which there is a chromatid gap, or a chromatid break, or an isochromatid gap but in which the chromosomes are otherwise structurally normal. These subdivisions are defined as follows:

(1) B_1 cells with a chromatid gap, characterized by a non-staining region in one chromatid.

(2) B_2 cells with a chromatid break, characterized by non-alignment of the broken ends.

(3) B_3 cells with an isochromatid gap, the non-staining region occurring at the same position in both chromatids.

Type C cells

These are cells showing any structural abnormality other than those characteristic of B types as defined above; in fact, they are cells showing chromosome types of structural change. There are two subdivisions as follows:

(1) C_u cells contain acentric fragments, dicentrics, or rings. Classically such abnormalities are regarded as unstable because they are liable to be lost or undergo further changes at cell division.

(2) C_s cells contain one or more morphologically abnormal chromosomes. They result from the re-arrangement of chromosome material following multiple breaks leading to inversions, deletions or translocations. Such cells are regarded as being stable at division.

In the original classification of Buckton *et al.* (1962) C_u cells were referred to as C_1 cells, and C_s cells as C_2 and C_3 cells. This modification is necessary as the designations C_1, C_2 and C_3 had already been introduced by Darlington & Upcott (1941) and used in a different sense.

The numbers of cells with each type of structural abnormality are shown in Tables 4 and 5. Also shown in the tables are the numbers of cells which had two or more different forms of structural abnormality. In Table 6 are recorded the proportions of cells with the major forms of structural abnormality. There is no evidence for any upward trend with age for any type of abnormality. Acentric fragments were the only structural abnormality in the great majority of C_u cells, and only 11 such cells were found to contain a dicentric chromosome, 5 in 2600 male cells and 6 in 3046 female cells. No cell was noted with a ring chromosome. A variety of different abnormal monocentric chromosomes were found in the C_s cells, but these have not been considered in detail.

Radiation exposure and structural abnormalities

An attempt was made to ascertain the history of exposure to radiation for medical diagnostic and therapeutic purposes for each member of the population. Nine males and twenty-two females claimed that they had never been exposed. For these males 16 out of 270 cells had type C abnormalities (5·93 %), while for the females 28 out of 660 cells had abnormalities in this

category (4·24 %). In neither instance does the proportion differ significantly from the proportion of cells with similar abnormalities in exposed subjects (males 3·73 %, females 2·98 %).

Several subjects were found to have had previous radiation exposure for therapeutic purposes. Three males had had limited superficial X-ray treatment for dermatological conditions and none showed any unusual proportion of type C cells. A fourth male had recently had palliative X-ray treatment for an advanced carcinoma of the bladder, and approximately 15 % of his cells showed structural abnormalities of the chromosome type. Two females had been exposed to radium for the induction of an artificial menopause, one in about 1924 and the other in 1942. Neither showed an undue proportion of type C cells.

Table 4. *The numbers of cells in males with different types of structural abnormality*

Age	Total cells	A_1	A_2	B_1	B_2	B_3	B_1+B_3	$B_1+B_2+B_3$	C_u	C_s	B_1+C_u	B_1+C_s	B_3+C_u	C_u+C_s	$B_1+B_2+C_s$
65–69	1140	986	72	26	3	6	0	0	14	26	2	1	2	2	0
70–74	750	666	48	12	2	5	1	0	4	12	0	0	0	0	0
75–79	330	285	17	7	1	5	0	1	5	9	0	0	0	0	0
80+	380	315	29	8	1	1	0	0	11	13	0	0	0	1	1
All ages	2600	2252	166	53	7	17	1	1	34	60	2	1	2	3	1

Table 5. *The numbers of cells in females with different types of structural abnormality*

Age	Total cells	A_1	A_2	B_1	B_2	B_3	B_1+B_1	C_u	C_s	B_1+C_u	B_1+C_s	B_3+C_u	C_u+C_s
65–69	1156	983	109	16	3	3	1	21	18	0	0	0	2
70–74	750	629	89	11	0	6	0	9	6	0	0	0	0
75–79	630	516	68	15	3	4	0	12	9	1	1	0	1
80+	510	409	63	11	2	5	1	10	7	0	0	1	1
All ages	3046	2537	329	53	8	18	2	52	40	1	1	1	4

Table 6. *The proportions of cells with different forms of structural abnormality as a percentage of the total cells counted**

	Males						Females					
Age	Total cells	B_1	B_2	B_3	C_u	C_s	Total cells	B_1	B_2	B_3	C_u	C_s
65–69	1140	2·5	0·3	0·7	1·7	2·5	1156	1·5	0·3	0·3	2·0	1·7
70–74	750	1·7	0·3	0·8	0·5	1·6	750	1·5	0·0	0·8	1·2	0·8
75–79	330	2·4	0·6	1·8	1·5	2·7	630	2·7	0·5	0·6	2·2	1·7
80+†	380	2·4	0·5	0·3	3·2	3·9	510	2·3	0·4	1·2	2·3	1·6
All ages	2600	2·3	0·3	0·8	1·6	2·5	3046	1·9	0·3	0·6	1·9	1·5

* Cells containing two and three different abnormalities are included in each of the relevant columns.

† Including one male with evidence of radiation-induced damage. Excluding this subject the proportions of C_u and C_s cells are respectively 2·6 and 3·1 %.

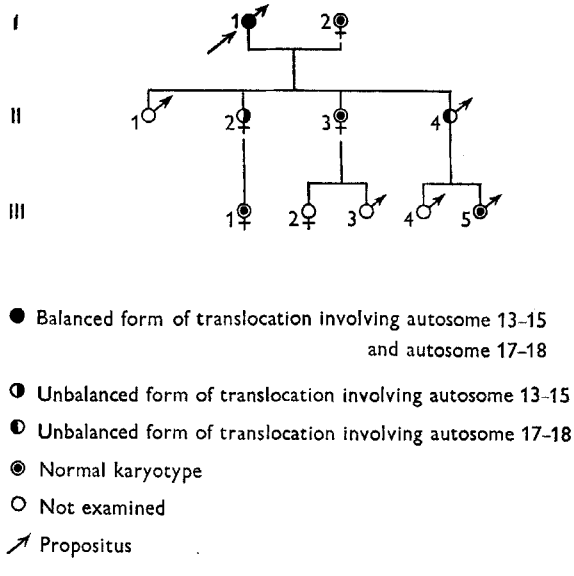
The frequency of individuals showing chromosome abnormalities or morphological variations in all cells

Eight out of 188 subjects (5 males and 3 females) had either an abnormal karyotype or a change which may be a variation of normal, a frequency of 4·2 %. In describing the findings in these individuals it is convenient to divide them into two groups. Group I is comprised of those

individuals with a structural chromosome modification which can only follow the production of breaks, leading to the transposition of material between chromosomes or from one part of a chromosome to another part of the same chromosome. Group II is comprised of individuals with a modification of a chromosome or chromosomes which may not need for its establishment the prior occurrence of chromosome breaks. However, it is not possible to exclude the occurrence of such breaks. For present purposes the chromosomes in group I are regarded as definitely abnormal, but for those in group II it is not possible yet to decide what is an abnormality and what is a normal variant.

Group I

(*a*) Subject W.B., a male aged 77 years. There was an abnormal chromosome in groups 13–15, the abnormality being the loss of most or all of the short arm. All or part of the material from the short arm appears to be translocated on to the short arm of a member of either pair 17 or pair 18, one member of these having satellites on its short arm (Pl. 1, fig. 1*a*). Studies have been made of the family of this subject (Text-fig. 1) and both theoretically expected unbalanced forms of the translocation have been found (II. 2, Pl. 1, fig. 1*b*: II. 4, Pl. 1, fig. 1*c*).



Text-fig. 1. Pedigree of W.B.

(*b*) Subject J.B., a male aged 73 years. There were 46 chromosomes present but no normal *Y* chromosome could be seen. However, an extra small metacentric chromosome was present, comparable in size to an autosome 19. The arms of this chromosome in terms of their apposition to one another and their somewhat fuzzy appearance strongly suggested the chromosome to be a metacentric *Y* (Pl. 1, fig. 2). The subject was only 5 ft. in height, had a hare-lip but did not appear to have any abnormality of sexual development. He had been married but had had no children, and had no living male relatives who could be examined.

Group II

(*a*) Subject H.M., a male aged 79 years. There was an unusually long short arm on one acrocentric autosome which was never seen to be satellited. In twelve cells the autosome so re-

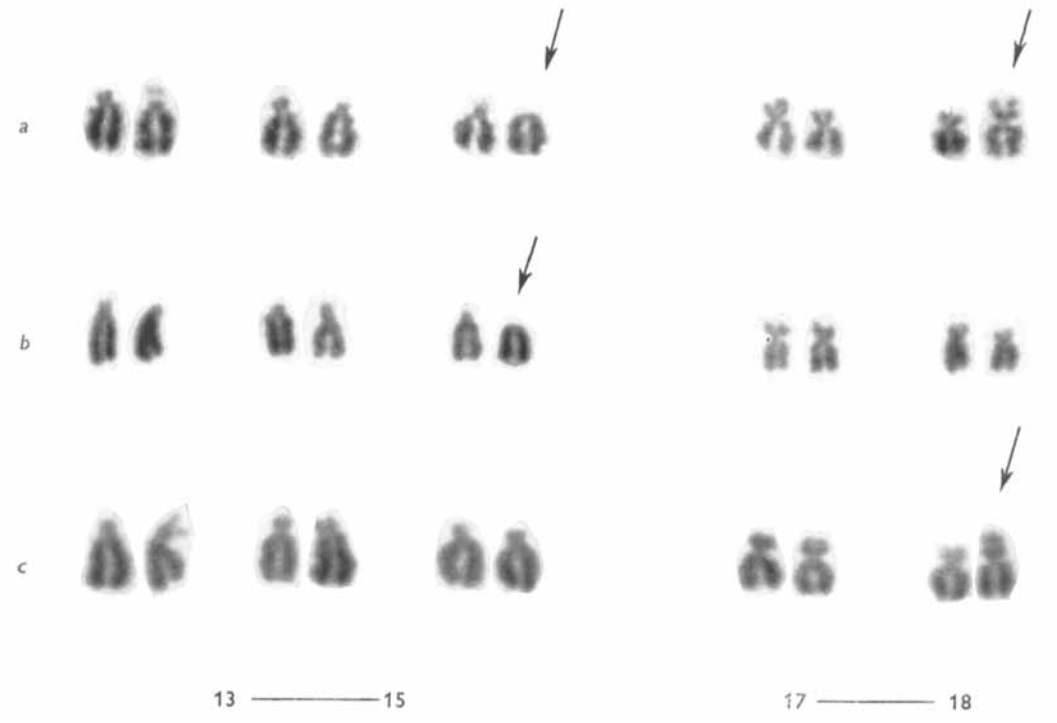


Fig. 1. (*a*) Autosome pairs 13–15 and 17–18 from subject W.B. (I. 1, Text-fig. 1). (*b*) Autosome pairs 13–15 and 17–18 from II. 2 (Text-fig. 1). (*c*) Autosome pairs 13–15 and 17–18 from II. 4 (Text-fig. 1).

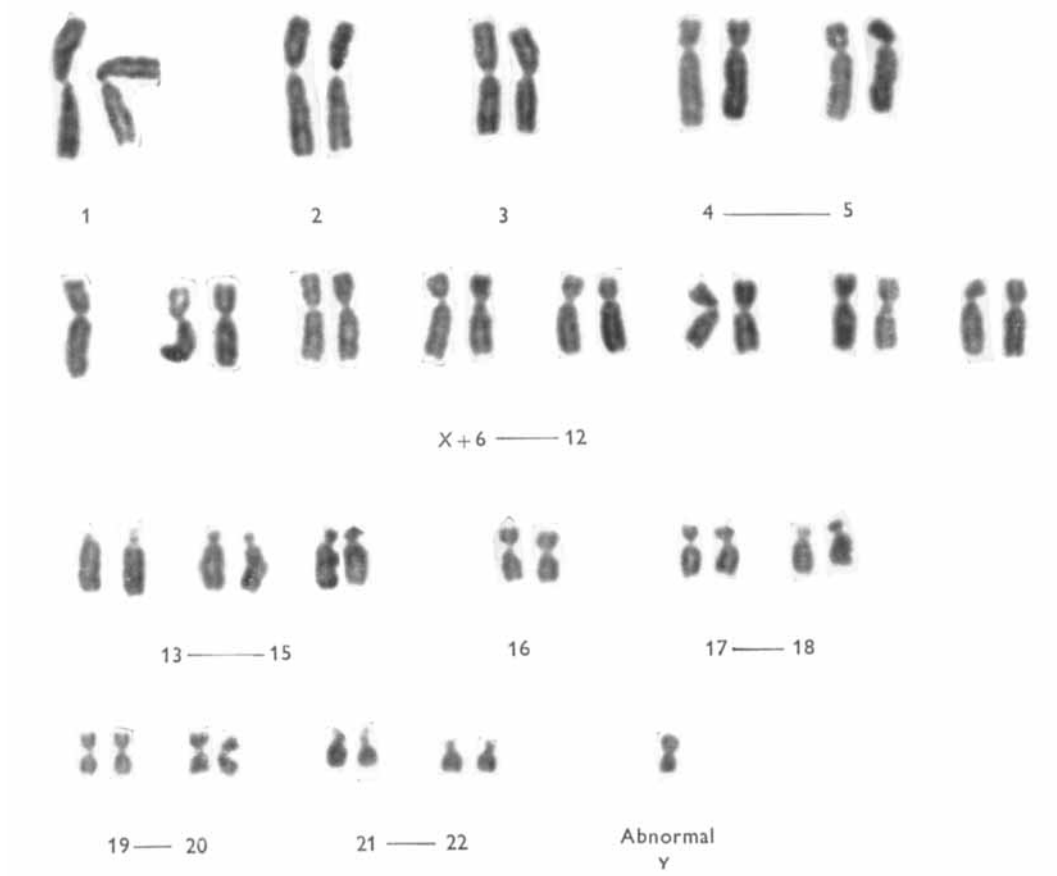
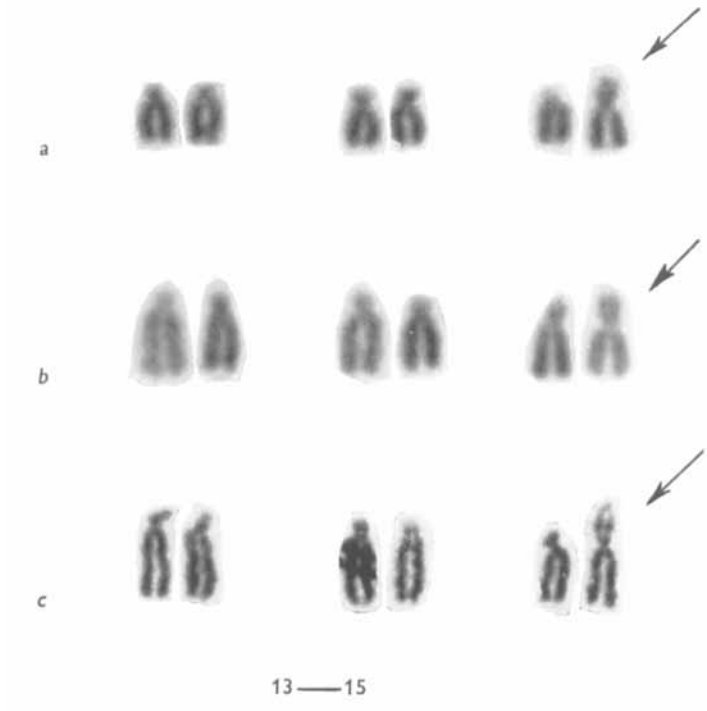


Fig. 2. Karyotype of subject J.B.



(*a*) Autosome pairs 13–15 from subject H.M. (I. 4, Text-fig. 2). (*b*) Autosome pairs 13–15 from II. 3 (Text-fig. 2). (*c*) Autosome pairs 13–15 from subject J.L.

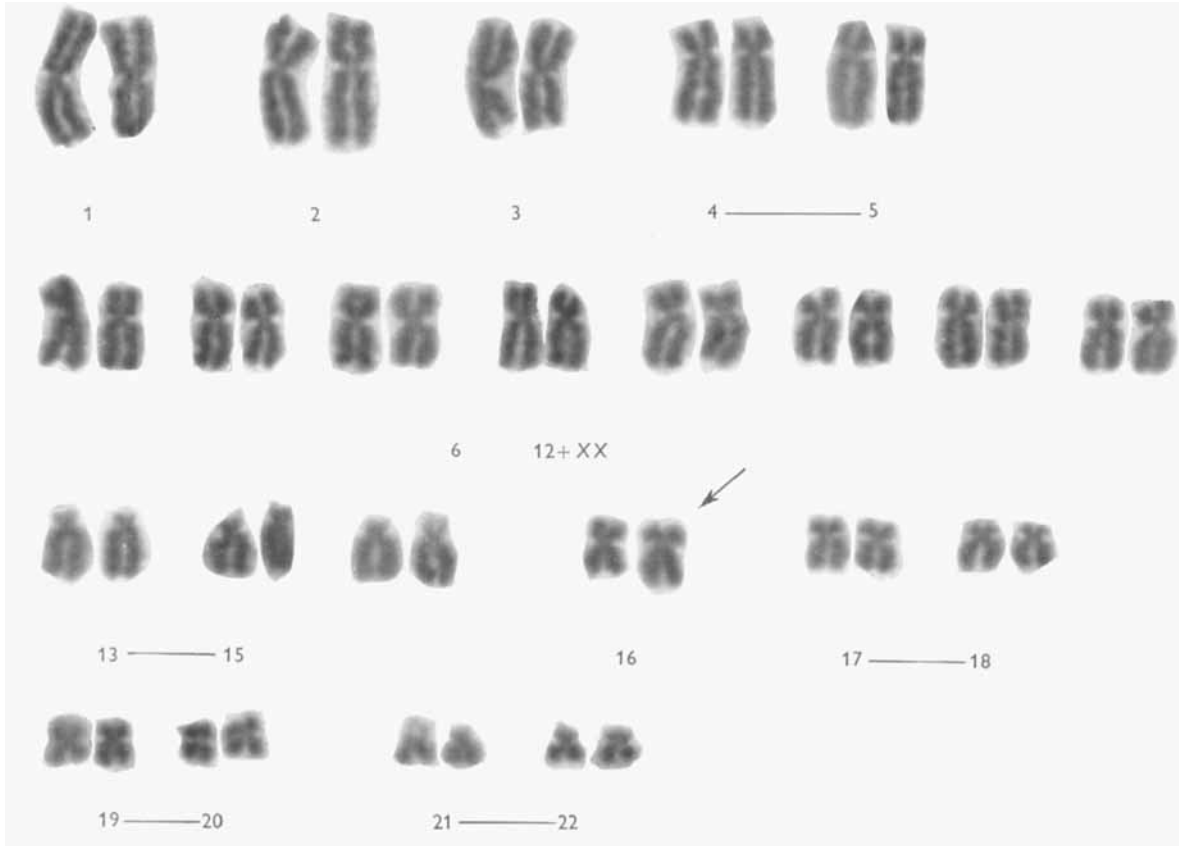


Fig. 1. Karyotype of subject H.H.

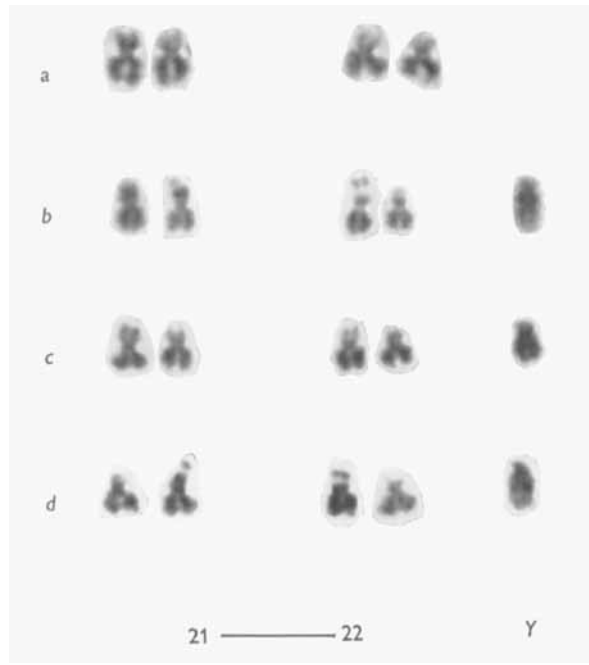
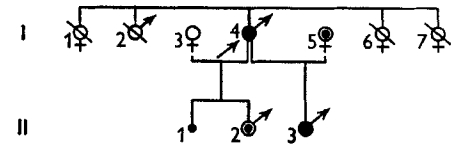


Fig. 2. (*a*) Autosome pairs 21–22 from subject C.M. (I. 2, Text-fig. 3). (*b*) Autosome pairs 21–22 and *Y* from subject E.P. (I. 3, Text-fig. 4). (*c*) Autosome pairs 21–22 and *Y* from I. 7 (Text-fig. 4). (*d*) Autosome pairs 21–22 and *Y* from II. 1 (Text-fig. 4).

sembled a medium-sized chromosome as to be indistinguishable. In the other eighteen cells the autosome was identifiable because the chromatids of the short arm had a somewhat fuzzy appearance and tended to lie in close approximation to one another superficially resembling the chromatids of the long arm of the *Y* chromosome (Pl. 2, fig. *a*). Studies have been made of the family of this subject (Text-fig. 2), the change also being found in II. 3 (Pl. 2, fig. *b*). No other change was detectable in the karyotype.

(*b*) Subject J.L., a female aged 79 years. There was an unusually long short arm on one long acrocentric autosome. In contrast with the findings in subject H.M. satellites were always present. In good quality cells a prominent secondary constriction was always present between the primary constriction and the secondary constriction normally associated with the satellite (Pl. 2, fig. *c*). No other change was detectable in the karyotype. No family studies have yet been possible.



● Long short arm autosome 13–15
◉ Normal karyotype
Dead not examined
○ Alive not examined
• Abortion
Propositus

Text-fig. 2. Pedigree of H.M.

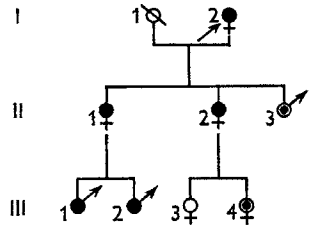
(*c*) Subject J.F., a male aged 69 years, and subject H.H., a female aged 69 years. In both subjects there was a difference in size between the homologues of pair 16. One homologue was appreciably larger than the other, being at least equal in size to the smallest medium-sized chromosome; the karyotype of a cell from H.H. is shown in Pl. 3, fig. 1. No other change was detectable in the karyotype. No family studies have been done, but it is known from the study of other subjects that this type of change is familial.

(*d*) Subject C.M., a female aged 73 years. There was an unusually long short arm on a small acrocentric autosome, no other change being present (Pl. 3, fig. 2*a*). Family studies have been done (Text-fig. 3), and a number of members carry the unusual chromosome.

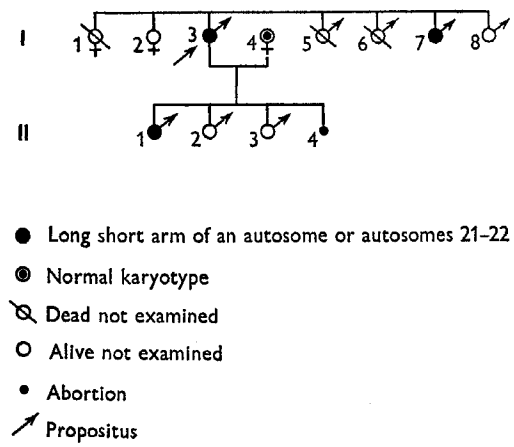
(*e*) Subject E.P., a male aged 67 years. All four small acrocentric autosomes presented different appearances (Pl. 3, fig. 2*b*). One had a very pronounced increase in the length of the short arm. In another the increase was present but not so marked and satellites were always present. In a third the short arms were of normal length but the satellites were unusually prominent. The remaining autosome had neither a long short arm nor unusually prominent satellites. Two members of his family have been studied (Text-fig. 4). In I. 7, one small acrocentric has a pronounced increase in the length of the short arm, the other three appearing normal (Pl. 3, fig. 2*c*). In II. 1, one small acrocentric chromosome shows some increase in the length of the short arm, satellites always being present (Pl. 3, fig. 2*d*).

DISCUSSION

Perhaps the most interesting findings of the survey refer to the number of subjects in a randomly chosen sample of individuals who had either an abnormal karyotype or changes which may provisionally be regarded as morphological variations. It is of further interest to note that, with the possible exception of subject J.B. with an abnormal *Y* chromosome, these abnormalities and variations were not associated with any obvious abnormalities of the phenotype. This was also true for the two children of W.B. who had the unbalanced forms of the translocation present in their father. It is convenient to discuss the findings in relation to the two groups of individuals described above.



Text-fig. 3. Pedigree of C.M.



Text-fig. 4. Pedigree of E.P.

Group I

It is now clearly recognized that subjects with a structurally abnormal chromosome or chromosomes occur in the human population. The first such abnormality to be found was a centric fusion involving a small and a large acrocentric autosome as reported by Turpin, Lejeune, Lafourcade & Gautier (1959). The chromosome number was 45, and the finding was made in a child with retardation of growth and multiple abnormalities of the spinal column. It is not known whether the association with the skeletal deformity was real or only fortuitous, and it is possible that this abnormality was the first of many which have been found to involve an autosome in the 21–22 group and another acrocentric chromosome (Penrose, Ellis & Delhanty, 1960; Polani *et al.* 1960). In 1960 Jacobs *et al.* published details of a woman with a structurally abnormal *X* chromosome, while in 1961 Böök, Santesson & Zetterqvist reported a translocation involving an autosome 3 and a medium-sized chromosome in an apparently normal female. Yet other abnormalities have been recorded (Moorhead, Mellman & Wenar, 1961; Edwards, Fraccaro, Davies & Young, 1962).

It is of fundamental interest that varied forms of structural heterozygosity occur in the human population and it is of considerable practical interest to determine their frequencies. Theoretically a proportion of the gametes of structural heterozygotes will carry unbalanced amounts of genetic material, which may be lethal to the zygote, or later to the embryo or to the

foetus, or may be compatible with post-natal life but possibly in association with some deformity or malformation. Obviously, therefore, if there are any large numbers of individuals in the population with structural heterozygosity, this could have some bearing on the causes of sterility, of abortions and stillbirths and of congenital malformations.

Group II

Classed in this group are those chromosomes with altered appearance which need not necessarily be due to structural re-arrangements of the types discussed in group I. In our experience four features are characteristic of these changes. First, they can be identified in every cell of good quality in a blood culture, while secondly, they may be presumed to be present in all tissues for they can also be identified in fibroblast cultures. Thirdly, they are familial, and fourthly, these changes seem confined to chromosomes which it is agreed possess a secondary constriction.

Secondary constrictions may be subterminal and separate a satellite from the main body of the chromosome (groups 13–15 and 21–22), or they may be more proximally situated (autosomes 1, 9 and 16). The changes that have been found involve the acrocentric chromosomes and pair 16. Changes have not been noted in pairs 1 or 9, but small variations in the size of one or other homologue of these latter pairs would be difficult to detect.

The changes in the acrocentric chromosomes involve the short arms, and appear to be related to their secondary constrictions. These changes may, of course, be small structural re-arrangements which are more easily detectable when they occur in the short arm. Alternatively, they may not be structural re-arrangements but represent inherited variations in the behaviour of the heterochromatic regions.

In pair 16 the characteristic finding is for one homologue to be larger than the other and to have a different arm ratio. The difference in size is variable, and for present purposes has only been regarded as significant if one homologue is at least as long as the shortest pair in group 6–12. It seems reasonable to postulate that the occasional variation in the size of autosome 16 is due to an inherited variation in the behaviour of its heterochromatic region. Again, however, the possibility of a structural re-arrangement cannot be dismissed.

The data reported in this communication have been obtained from the first of a series of studies which are being undertaken to define the frequency of abnormalities and variations in a random sample drawn from a general human population. As they stand the data are as yet too limited and too unrepresentative of the population as a whole to permit even the drawing of interim conclusions about the population at large. The findings do indicate, however, that chromosome polymorphism and abnormalities are perhaps commoner in man than had been suspected. It is hoped that within a comparatively short time it will be possible to define more precisely the frequency of chromosome aberrations and ultimately the extent to which these may contribute to human disease.

SUMMARY

The results are reported of chromosome studies on leucocyte cultures from a randomly chosen group of 189 subjects (87 males and 102 females) of ages 65 or more. Particular attention is paid to the distribution of chromosome counts, the frequency of cells with chromosomes showing a structural abnormality, and the frequency of sub ects showing a consistent abnormality or variation in their karyotype.

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