No definite relationship was seen between the number and duration of the lesions in any particular patient and the total number of nasal swabs from him which yielded *Staph. pyogenes*. Moreover, lesions were frequently present when the nasal swab was negative, and vice versa. In fact, we obtained no useful information from the nasal swabs after we had made the preliminary survey of the types of staphylococci involved in the outbreak.

Clinical

The subsequent course of the epidemic is shown by the following numbers of lesions which appeared in successive months:

1959	{	November 1 to 15	39	}	41	1960	{	January	5	}
		" 15 to 30	2					February	5	
		December	8							

Later Events

By February, 1960, conditions had so much improved that on February 29 various measures were relaxed. It was arranged that the use of intranasal naseptin cream, sponging in 1 in 5,000 hibitane solution after bathing, and nasal swabbing should be continued only in respect of patients with lesions. Members of the staff and patients without lesions were to be excluded from these measures.

There followed an immediate increase in the number of new lesions, which totalled 24 during March. We therefore reintroduced naseptin cream and sponging with hibitane for all the patients. During April nine new lesions occurred, during May 10, and during June only 3. In June, naseptin cream was again restricted to patients who had lesions, being applied while the lesions were present and for a few days afterwards. A gradual increase of new lesions was then seen, reaching a peak in August, after which the numbers fell spontaneously without any further action on our part. (This peak may have been wholly or in part a seasonal phenomenon associated with the warmer weather.)

The numbers of new lesions in successive months from June, 1960, to the time of writing were: June, 3; July, 8; August, 25; September, 18; October, 9; November, 9; December, 5; January (1961), 2; February, 5; March, 6.

Discussion

It is perhaps of some general interest that the epidemic was checked immediately without any form of segregation of the patients or staff, or of their belongings, and without isolation or barrier-nursing techniques. We cannot assess the relative values of the different measures we used, but our experience in March, 1960, led us to believe that the use of intranasal naseptin cream and sponging with hibitane 1 in 5,000 solution were playing an important part in holding the position at that time. We have seen no case of sensitization nor any other adverse effect from the use of these substances. It is regrettable that small numbers of new lesions continue to appear, but most of them are now papules or furuncles which heal in a few days, and only three boils have been seen during the first quarter of 1961. When one considers the nature of the unit these facts are not too disappointing.

Summary

An epidemic of cutaneous staphylococcal infections due to phage types 3B/3C+ and 80 occurring in a unit housing 30 mentally subnormal children was brought

rapidly under control without any form of segregation, isolation, or barrier-nursing. The methods used are recorded.

We should like to pay a sincere tribute to the work of the nursing staff, particularly the sister-in-charge, Miss Mary Huston, who applied our suggestions with enthusiasm under conditions that could hardly have been less promising.

THE TRIPLE-X SYNDROME

CLINICAL, PATHOLOGICAL, AND CHROMOSOMAL STUDIES IN THREE MENTALLY RETARDED CASES

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During the course of a survey of nuclear sex in a mental deficiency hospital, five patients with double sex-chromatin masses in their buccal mucosal cells were found (Ferguson-Smith, Handmaker, and Hill, in preparation). Two of these were phenotypic males with the clinical features of Klinefelter's syndrome, who were shown to have a chromosome number of 48 with an XXXY sex-chromosome constitution (Ferguson-Smith, Johnston, and Handmaker, 1960). The other three, who form the basis of this report, were females with 47 chromosomes.

Methods

The chromosome counts were performed on both bone-marrow and peripheral blood cells. The former technique is given elsewhere (Ferguson-Smith, Johnston, and Weinberg, 1960). The latter is based on a method originally described by Osgood and Brooke (1955) and modified by a number of workers. Thirty millilitres of the patient's blood are drawn into a heparinized syringe and placed in 10-ml. amounts in three sterile tubes, to each of which 0.25 ml. bacto-phytohaemagglutinin (Difco) is added; these are put in ice-water for one hour. After centrifuging for 10 minutes at 300–400 r.p.m. the opalescent plasma containing leucocytes is added in 3-ml. amounts to 2-oz. sterile prescription bottles containing 5 ml. of culture medium (NCTC 199 Difco). These are incubated for 66–80 hours. The cultures are treated in succession for the last two hours of incubation, with deacetylmethylcolchicine ("colcemid") to a final concentration of 1 µg./ml. After centrifugation at

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1,000 r.p.m. for five minutes the supernatant is discarded, and the deposit is incubated with 7 ml. of 1.12% sodium citrate for 15 minutes. Following centrifugation, 7 ml. of 50% acetic acid is used as a fixative. The deposit, with only a small quantity of the supernatant left after further centrifugation, is used to make air-dried smears as for blood films. These are stained with 2% aceto-orcein in 65% acetic acid, dehydrated, cleared, and mounted as usual.

Dr. John Money reported (May 21, 1960) that her I.Q. was less than 46 on the Wechsler intelligence scale for children. On the revised Stanford-Binet scale (Form L), an I.Q. of 18 was obtained. She can comprehend some of the events around her and can refer them appropriately, so that she will co-operate in most of the simple demands made of her.

Certain features suggest toxoplasmosis, particularly the choroidoretinitis in association with mental defect and epilepsy. The Sabin dye test was reported positive only at 1:64 dilution. The absence of intracranial calcification, and the relatively normal condition at birth, make this diagnosis uncertain.

Case 2

This patient was born on October 24, 1934. The father at this time was aged 33 and the mother 17. The latter is now confined to a State institution with a diagnosis of paranoid schizophrenia. A maternal aunt and uncle, in addition to a paternal aunt, have schizophrenia, while a maternal uncle is said to have general paralysis of the insane. There are four male sibs, all but one of whom are probably half-brothers. The three eldest have children. Both parents have normal nuclear sex.

The patient was admitted to a mental hospital in 1948 because of severe retardation. Her menstrual history is not well documented and her last menstrual period is not known, but she is stated to have periods at irregular intervals. Her general health is otherwise good.

Examination shows a severely retarded negro female of normal proportions (Fig. 2). She is hyperactive and cannot carry on a conversation. The head is small, with a high-arched palate and malocclusion of the teeth. The incisors are peg-shaped and she has marked gingivitis. Both pupils are irregular and react poorly to light and accommodation. There is a right corneal opacity. The tone is increased on the left, with increased reflexes in the left leg. Both plantar responses are flexor. The breasts are normally developed.

FIG. 4.—Case 1. Palmar aspect of the hands showing incurved fifth digits, one interphalangeal skin crease of fifth right digit, and left transverse palmar crease.

FIG. 1

FIG. 2

FIG. 3

FIG. 1.—Case 1, aged 14 years. FIG. 2.—Case 2, aged 26 years. FIG. 3.—Case 3, aged 39 years.

Urinary oestrogens were assayed by the method of Brown (1955), and urinary 17-ketosteroids by the method of Callow, Callow, and Emmens (1938).

Case Histories

Case 1

The patient was born on August 27, 1945. Her father was 33 and her mother 29 years of age at this time. There is one half-brother, aged 20, who has an infant daughter. The nuclear sex of the mother is normal.

She was admitted to a mental hospital in 1956. Her physical development has been normal, and she had her first and only recorded menstrual period in March, 1960, two months prior to laparotomy. Since 1949 she has had occasional seizures, usually in association with a high temperature.

Examination shows an unusually attractive but mentally retarded adolescent white female (Fig. 1). Her head is small, and she has a high-arched palate. The fingers are short and the little fingers are incurved, with only one interphalangeal crease on the right, while the left hand shows a transverse palmar crease (Fig. 4). There is bilateral optic atrophy and choroidoretinitis. The nervous system is otherwise normal. The breasts are normally developed. The external genitalia are normal and the vagina admits two fingers. The cervix is nulliparous, and the uterus is at the lower limit of normal in size. The adnexa are not palpable. Pubic and axillary hair are normal in amount and development. Systematic examination is otherwise normal. Radiological examination revealed a normal bone age and a brachycephalic skull.

Axillary and pubic hair are normal, as are the external genitalia. The vagina admits one finger. The cervix is infantile and the uterus normally formed but small. The adnexa are small and free. Otherwise, physical examination shows nothing of note. A skull radiograph showed brachycephaly. The cerebrospinal fluid was normal and the *Treponema pallidum* immobilization test was negative.

Dr. John Money reported that it was not possible to obtain a value for her I.Q., her level being that of a mentally defective person of very low grade. In addition, he thought that she was having hallucinations and that the primary defect might be a congenital psychosis.

Case 3

This patient was born on August 27, 1921. Her mother was aged 18; nothing is known of the father. There are four living and apparently healthy half-sibs, and two others died in infancy for reasons now unknown. Three sibs of the mother are mentally retarded and two cousins have congenital deformities. The nuclear sex of the mother is normal.

She was admitted to a mental hospital in 1936. She menstruates regularly and her last menstrual period was about seven days prior to laparotomy.

Examination shows a moderately severely retarded white female of normal proportions who talks volubly (Fig. 3). There is good breast development, with normal distribution and amount of pubic and axillary hair. The external genitalia are normal. Pelvic examination reveals a normal vagina. The cervix and uterus are of normal size and in normal position. The adnexa are normal.

Her I.Q. on the Stanford-Binet scale was less than 31 (July 27, 1960), and her behaviour like that of a 4-year-old child.

Results

Chromosome Analysis

The majority of cells from both blood and bone-marrow in each patient showed 47 chromosomes (Table I). Detailed analysis of the 22 complete plates showed that the extra chromosome lay in Group III (Chromosome Nos. 7-12 and X) and fitted best into a triple-X pattern (Fig. 5). Five of the cells with fewer than 47 chromosomes were analysed. Different chromosomes were missing in each, suggesting loss of chromosomes by cell breakage during preparation. No evidence of chromosomal mosaicism was found.

TABLE I.—Results of Chromosome Analyses

		No. of Cells with Chromosome Count of:							Total	Plates Analysed			
		43	44	45	46	47	48	49		47	46	45	Total
Case 1	Blood Marrow	4	10	12	12	76	1	8	114 } 123	5	1		6
" 2	Blood Marrow	3	7	7	10	41		1	69 } 77	10	1		11
" 3	Blood Marrow	5	6	9	16	64	1		101 } 139	7	2	1	10
		1		3	4	30			38 }				

Special Investigations

The results of some of the special investigations are recorded in Tables II, III, and IV.

TABLE II

	Case 1	Case 2	Case 3
Ovary size (cm.), L.	2.5 × 1.0 × 0.8	3.5 × 1.8 × 1.2	3.5 × 1.8 × 1.1 (7.5 c.cm.)
" " " R.	3.0 × 1.5 × 1.0	3.0 × 2.5 × 1.2	3.0 × 2.2 × 2.1 (9.0 c.cm.)
Uterus size (cm.)	7.5 × 4.5 × 2.5	7.0 × 3.8 × 3.0	" Within normal " (not measured)
External genitalia	Normal	Normal	Normal
Secondary sex characteristics	"	"	"
Ovarian histology, L.	Scanty follicles, undifferentiated stroma	Old corpus luteum, scanty follicles	"
Ovarian histology, R.	Scanty follicles, follicular cyst	Scanty follicles	Recent corpus luteum
Endometrium	Non-secretory	Non-secretory	Secretory

TABLE III.—Urinary Hormone Excretion/24 Hours

	F.S.H. Mouse Units	17-Keto-steroids mg.	Oestrone µg.	Oestriol µg.	Oestradiol µg.	Total Oestrogens µg.
Case 1	(a) <6.6 (b) <6.6 <13.2 <26.4 <52.8	2.63 5.36	5 3	16 10	3 4	24 17
" 2	(a) <6.6 <13.2 <26.4 <52.8 (b) <6.6 <105.6	9.6 6.7	1 1	3 2	1 1	4 2
" 3	(a) <13.2 <52.8 <105.6 (b)	1.8 2.0	10	11	6	27

FIG. 5.—Karyotype of a peripheral blood cell in Case 2. $2n+1=47$ (XXX).

At the time these studies were begun (May, 1960) only one other case with similar features was known. This case, reported by Jacobs *et al.* (1959), was that of a woman aged 35 with secondary amenorrhoea since the age of 19. She had double chromatin-positive nuclear sex and a somatic chromosome number of 47 with sex-chromosome constitution of XXX. In view of the gynaecological findings in this patient, it was decided to investigate the genital system of our patients as adequately as possible.

At laparotomy the internal genitalia were examined carefully, wedge sections were removed from the ovaries for histological examination, and endometrial biopsy material was

obtained. In each case the uterus was normal or somewhat smaller than normal (Table II), the Fallopian tubes were unremarkable, and there was no evidence of abnormal Wolffian derivatives. In Case 1 the ovaries were small (Fig. 6 and Table II), but in Cases 2 and 3 they were of normal size (Fig. 7).

On histological examination the ovarian stroma in Case 1 was poorly differentiated on both sides, and primordial ova were scanty, although a few maturing Graafian follicles were identified on the right (Fig. 8). Histological evidence of previous ovulation was not

found in this patient. Ovarian follicles were also scanty in Case 2, despite normal-appearing ovarian stroma and evidence of an old corpus luteum. In Case 3 ova were seen in numbers consistent with the age of the patient, a recent corpus luteum was identified, and the ovarian stroma appeared normal (Fig. 9).

FIG. 6.—Case 1. Internal genitalia exposed at laparotomy. Small uterus, normal tubes, and hypoplastic ovaries. Right ovary almost entirely replaced by a simple follicular cyst.

FIG. 7.—Case 2. Internal genitalia exposed at laparotomy. Small uterus, normal tubes, and normal-sized ovaries.

FIG. 8.—Case 1. Maturing Graafian follicle in right ovarian biopsy material. (Haematoxylin and eosin. $\times 85$.)

FIG. 9.—Case 3. Normal ovarian stroma with primordial ova in left biopsy material. (Haematoxylin and eosin. $\times 135$.)

FIG. 10.—Case 3. Secretory endometrium in endometrial biopsy material at approximately 19th day of cycle. (Haematoxylin and eosin. $\times 203$.)

In Case 3 an endometrial curetting obtained at the time of laparotomy contained insufficient tissue for diagnosis. An additional endometrial biopsy obtained on this patient three months after laparotomy on the 19th day of the cycle revealed an early secretory endometrium (Fig. 10), diagnosed as day-16 stage according to the criteria of Noyes *et al.* (1950) but nevertheless confirming continued ovulation. Endometrium obtained at laparotomy in Cases 1 and 2 showed no secretory changes.

A prominent feature of the histological material in all cases was the high incidence of two sex-chromatin masses in cell nuclei.

Discussion

Identification of the X Chromosome

We interpret the chromosome analyses in these three patients as being consistent with a trisomic-X sex-chromosome constitution, because in each case the extra chromosome belongs to a group of three pairs of chromosomes (Nos. 6, X, and 7) in which the X chromosome is normally found. The small differences in size and centromere position between these three pairs of chromosomes make it impossible to identify with absolute certainty the three X chromosomes in any given cell. However, circumstantial evidence that this is the correct interpretation may be derived from the abnormal sex-chromatin findings, for it has been the experience of most investigators that patients with a distinct nuclear-sex anomaly always have an abnormality of the sex-chromosome constitution. Although the origin of the sex-chromatin body must still be considered unsolved, available evidence relates it directly to part of a single X chromosome (Ferguson-Smith, 1960; Ohno and Makino, 1961).

We therefore feel justified in including in our list of known cases of the triple-X syndrome (Table IV) two cases each of which has a somatic chromosome number of 47 and double chromatin-positive nuclear sex, but

which were not originally reported as triple-X cases. The patient of De Carli *et al.* (1960) was referred to as a "paramongol," and showed slanting palpebral fissures; the additional chromosome was interpreted as an extra autosome in the 6-12 group, possibly homologous with the tenth or eleventh pair. The patient of Sandberg *et al.* (1960) was also studied because of apparent mongoloid facies; in this case the abnormality was interpreted as trisomy of chromosome No. 6. It is possible that in these two cases the cytological findings are complicated by structural abnormalities of an X chromosome—that is, a deletion in the first instance and a translocation in the second instance. The recorded percentages of cells showing sex-chromatin bodies (Table IV) may possibly support such an explanation. For these and other reasons recorded elsewhere (Jacobs *et al.*, 1960a; Ferguson-Smith, 1961a), we have included for purposes of comparison these two patients among the examples of the triple-X syndrome.

Clinical Features of the Triple-X Syndrome

It should be emphasized, before attempting to assess the phenotypic effect of the extra X chromosome, that the patients listed in Table IV, with three exceptions, were ascertained on account of mental defect. The sample is therefore a biased one. A completely satisfactory assessment of the syndrome will not be possible until the results of a large-scale nuclear-sexing survey of the general population are available. Such a survey would require a far larger sample than, for example, that of Moore (1959), who did not detect any triple-X cases in his survey of 1,804 newborn girls. The increased frequency of the condition among mentally defective patients is illustrated by the three cases described here, which were detected in a sample of 827 (0.36%) females at one mental deficiency hospital (Ferguson-Smith, Handmaker, and Hill, in preparation).

The additional X chromosome does not appear to have a consistent effect on sex differentiation. In only

TABLE IV.—Known Triple-X Cases

Age	I.Q.	Maternal Age	Paternal Age	Sibship Position	Menstrual History	Pregnancies	Single Sex Chromatin	Double Sex Chromatin	Drumstick Polymorphs	Height (cm.)	Lower Segment	Upper Segment/Lower Segment	Span (cm.)	Special Clinical Features	Case No.	
35	? N	41	40	3/3	14-19 yrs. only	0	57	14	8/500	176	—	—	—	—	—	Jacobs <i>et al.</i> (1959)
21	M.D.	—	—	—	N	0	41	48	11/500	152	79	0.90	158	—	3	Jacobs <i>et al.</i> (1960b)
35	70	42	42	4/5	N	4	38	14	—	168	84	1.00	164	—	—	Stewart and Sanderson (1960)
19	70	34	39	—	N	—	49	8	—	167	—	—	—	"Paramongol"	—	De Carli <i>et al.</i> (1960)
21	40	41	49	4/?	N	—	?	70	5/100	172	—	—	—	Mongoloid facies. Webbed neck	—	Sandberg <i>et al.</i> (1960)
30	50	28	30	4/8	N	1	43	30	—	156	80	0.94	156	—	1	} Fraser <i>et al.</i> (1960)
39	58	35	—	5/5	Irreg.	0	50	25	—	178	84	1.10	170	—	2	
61	38	40+	40-50	10/11	N	0	35	33	—	151	72	1.11	147	—	3	
73	50	—	—	2/3	—	0	33	30	—	156	77	1.02	154	—	4	
52	M.D.	34	33	6/7	13-17 yrs. only	0	—	—	—	155	78	0.92	156	—	S.S.	} Jacobs, personal communication
47	M.D.	40	46	11/11	N	0	—	—	—	147	—	—	145	Acute encephalitis aet. 12 yrs.	J.H.	
18	? N	33	34	4/4	N	0	—	—	—	156	71	1.14	156	—	N.B.	
New born	? N	34	37	3/3	—	—	—	—	—	—	—	—	—	Normal child	B.R.	
23	M.D.	21	27	2/3	Two only	0	22	10	2/374	147	—	—	—	Spastic quadriplegia	M.S.	Hamerton <i>et al.</i> , personal communication
18	63	38	37	4/4	N (Irreg.)	0	54	8	"High" incidence	156	79	0.98	160	Nil	J.B.	Penrose, personal communication
15	46	29	33	2/2	? N	0	44	40	6/132	166	89	0.86	166	"Mongoloid" palms	1	} Present communication
26	<20	17	33	1/1	Irreg.	0	38	50	6/123	163	85	0.91	166	—	2	
39	31	18	—	—	N	0	41	37	6/115	162	86	0.90	159	—	3	

four of the cases recorded in Table IV were the internal genitalia examined. In two cases the uterus was noted to be small, but this is unlikely to be significant. There is clearly no consistent abnormality of the ovary. In three cases the number of follicles was reduced, and in one case (Case 1) the ovaries were small and the ovarian stroma was poorly differentiated. In three cases out of 16 whose menstrual history is documented, secondary amenorrhoea is present, and in three others the periods are irregular. Of the 10 who have had a normal menstrual history, two are of proved fertility, despite the fact that they were detected during surveys of mental deficiency hospitals. Furthermore, our three patients are potentially fertile, since corpora lutea were found in two and normally maturing ova identified in the other.

The body proportions of these patients as assessed by height, span, and upper-segment/lower-segment ratios are unremarkable, and show no evidence of the disproportion noted in the XXY (Stewart *et al.*, 1959) and the triple X/Y (Ferguson-Smith *et al.*, 1960) cases, nor any striking evidence of short stature as in the XO cases of Turner's syndrome.

The only other clinical signs of note are the questionable mongoloid features which are present in three cases, and the webbing of the neck, which was found in one case. As webbing of the neck is a well-known feature of Turner's syndrome and has also been noted in one triple X/Y case (Ferguson-Smith *et al.*, 1960), it is of interest that it has been found in a third type of X-chromosome anomaly.

It is exceedingly difficult to compare the results of nuclear-sex determinations from different centres, as no two investigators have exactly the same criteria for counting. In the three cases reported here the numbers of "drumstick polymorphs" appear to be increased, the value for normal female blood smears being about 2-3%; no cells were identified with two drumsticks. In the buccal smears of our patients, double chromatin-positive nuclei were seen as frequently as single chromatin-positive nuclei, together giving a count twice the usual value for normal female smears.

The significance of the consistently low values for urinary 17-ketosteroids recorded in Table III remains unexplained. It is of interest that similar findings are noted by Carr *et al.* (1961) in their report on two mentally defective females with an XXXX sex-chromosome constitution.

Origin of the Triple-X Chromosome Abnormality

The data recorded in Table IV suggest that, as in mongolism, parental age may play a part in the aetiology of the condition.

The average maternal age of the 11 British patients is 35.1 years compared with a mean of 28.7 years (Penrose, 1961). The average age of eight of these fathers was 37 years compared with a mean of 32.2. The difference between mean of sample and expected = $35.1 - 28.7 = 6.4$ years. $t = \frac{6.4}{1.89} = 3.39$ with 10 degrees of freedom. This is significant at the 0.01 level.

Similarly for the paternal age: The difference between mean of sample and expected = $37 - 32.2 = 4.8$ years. $t = \frac{4.8}{2.07} = 2.32$ with 7 degrees of freedom. This is significant only at the 0.1 level.

If the mean increase in age of the fathers is corrected for regression on that of the mothers, it becomes only -0.2 year, which is clearly negligible. This suggests that the apparent increase in the father's age is only a reflection of that of the mother. However, the opposite interpretation—that the apparent increase in the mother's age is only a reflection of the father's—seems less plausible. The increase in the mother's age after correction for regression on the father's becomes 3.2 years, which is not significant in such a small sample.

The trend to a higher than average maternal age is supported by the records on sibship position, which indicate that out of 14 families, the propositus was the last-born child in eight and the second last in five. The accumulation of data from a greater number of cases from the general population is clearly desirable.

With regard to the origin of sex-chromosome aneuploidy in general, cytological studies of human meiosis in testicular material (Ford and Hamerton, 1956; Kodani, 1958; Ferguson-Smith, 1961b) raise the possibility that precocious disjunction of the sex chromosomes at the first meiotic division is one of the most likely explanations. Gametes bearing both sex chromosomes, or neither, may also arise by failure of the sex chromosomes to pair at the first meiotic division, or by non-disjunction of the sex bivalent; however, it is impossible at present to demonstrate which one of these various explanations is correct. So far as the triple-X patients are concerned, the parental age data might be interpreted as evidence in favour of the defect arising during oogenesis. On the other hand, the existence of XO/XXX mosaicism (Jacobs *et al.*, 1960b) might well be interpreted as good evidence for another possible mechanism—namely, mitotic non-disjunction in the zygote with elimination of the XO cell line.

As most cases of triple-X syndrome are fertile, or potentially so, theoretically the normal process of chromosome segregation during meiosis should produce equal numbers of ova containing one and two X chromosomes. When fertilized by normal sperm the abnormal XX ova will give rise to XXX and XXY individuals.* As the nuclear sex of all the offspring and all the parents of the triple-X cases so far tested has been normal, there is no evidence that this type of familial transmission occurs. It may be that in the fertile triple-X cases there is selection in favour of normal ova or zygotes.

Conclusion

No consistent clinical pattern can be distinguished in the examples of the triple-X syndrome at present available. While a high proportion of cases may be seen on account of mental or gynaecological disturbances, there remains the possibility that cases exist in the general population without recognizable clinical abnormality, and detectable only by nuclear-sex determination and chromosome analysis.

Summary

Clinical and chromosomal studies are reported in three mentally defective individuals with the triple-X syndrome. Histological evidence of normally maturing

*Where the two X chromosomes are those that were involved in primary non-disjunction in the mother, this situation is correctly referred to as secondary non-disjunction as originally defined (Bridges, 1913). Where both the X chromosomes are not those that failed to disjoin primarily, the trisomy is not strictly the product of secondary non-disjunction but of normal segregation.

ova in each, and the additional finding of corpora lutea in two, indicate that these patients are potentially fertile. Examination of clinical and laboratory data of all similar cases at present available fails to reveal a characteristic syndrome related to the presence of an extra X chromosome and distinguishable on clinical grounds.

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STUDY OF CHROMOSOMES IN HUMAN
LEUKAEMIA BY A DIRECT METHOD

BY

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Since the first reports (Ford *et al.*, 1958; Nowell *et al.*, 1958) of chromosome studies in human leukaemia, great interest has centred on this problem. The findings of different workers have varied considerably, especially with regard to acute leukaemia, where numerical and/or structural abnormality was found in one of the three cases studied by Ford *et al.* (1958), in four of the five cases reported by Baikie *et al.* (1959), and in three of the five cases recorded by Sandberg *et al.* (1960). On the other hand, normal findings have been reported by Nowell and Hungerford (1960a) in three cases of acute leukaemia, by Bayreuther (1960) in five cases, while Tough *et al.* (1961) recorded no abnormality (apart from the trisomy of autosome 21) in five cases of acute leukaemia occurring in mongols.

There is more agreement regarding the situation in chronic leukaemia, the majority of reports of which agree that there is probably no numerical abnormality. As regards structure, however, Nowell and Hungerford (1960a, 1960b) reported that in seven cases of chronic myeloid leukaemia one of the smallest autosomes (21 pair, Denver classification), was much reduced in size. This observation by the Philadelphia workers has been confirmed by the Edinburgh group (Baikie *et al.*, 1960a; Tough *et al.*, 1961), who found this minute chromosome, termed by them the Ph¹ chromosome, in 13 out of 18 cases of chronic myeloid leukaemia.

We report here a study of 16 cases of leukaemia in which a direct non-culture technique of preparation (Kinlough *et al.*, 1961) has been employed. In this way some of the difficulties of interpretation of culture results (Ford and Mole, 1959) have been avoided. In this study an abnormality of chromosome number has been found in two of the eight cases of acute leukaemia studied, while the presence of the Ph¹ chromosome has been confirmed in two of the four cases of chronic myeloid leukaemia.

Methods

Throughout this investigation a deliberate attempt was made to obtain samples for study, either from bone-marrow or from peripheral blood, before any antileukaemic treatment had been given. This was successful in all but three instances.

The methods employed have been previously reported (Kinlough *et al.*, 1961). In the earlier part of this work colchicine (0.1 mg./kg. body weight) was injected intravenously one hour before bone-marrow aspiration. (This was felt to be permissible in leukaemic cases.) Later it has been found that the use of E.D.T.A. (dipotassium (ethylene-dinitrilo) tetra-acetate) as an anticoagulant into which the marrow or blood cells are received renders the use of colchicine unnecessary, since E.D.T.A. appears to have a marked colchicine-like