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.

We gratefully acknowledge the co-operation of Dr. Harry G. Butler, director of medicine, and the members of the medical and nursing staff of Rosewood State Training School, Maryland, U.S.A. Our thanks are also due to Miss Patricia Jacobs, Professor L. S. Penrose, Mr. J. L. Hamerton, and Dr. E. Poli for permission to quote the results of unpublished observations; to Dr. John Money for psychological reports; and to Yvonne Phillips Hajda and Bonny Morgan Lewis for technical assistance.

REFERENCES

- Bridges, C. B. (1913). Non-disjunction of the Sex Chromosomes of *Drosophila*. *J. Exper. Zool.*, **15**, 587.
- Brown, J. B. (1955). A Chemical Method for the Determination of Oestriol, Oestrone, and Oestradiol in Human Urine. *Biochem. J.*, **60**, 185.
- Callow, Nancy H., Callow, R. K., and Emmens, C. W. (1938). Colorimetric determination of Substances containing the Grouping CH₃CO in Urine Extracts as an Indication of Androgen Content. *Ibid.*, **32**, 1312.
- Carr, D. H., Barr, M. L., and Plunkett, E. R. (1961). An XXXX Sex Chromosome Complex in Two Mentally Defective Females. *Canad. med. Ass. J.*, **84**, 131.
- De Carli, L., Nuzzo, F., Chiarelli, B., and Poli, E. (1960). Trisomic Condition of a large Chromosome in a Woman with Mongoloid Traits. *Lancet*, **2**, 130.
- Ferguson-Smith, M. A. (1960). Nuclear Sex and the Sex Chromosomes. *J. Chron. Dis.*, **12**, 203.
- (1961a). Trisomy of a Large Chromosome. *J. Amer. med. Ass.*, **175**, 414.
- (1961b). Sex Chromatin Anomalies in Mentally Defective Individuals. *Acta Cytologica* (in press).
- Handmaker, S. D., and Hill, R. N. The Incidence of Nuclear Sex Anomalies in a Mental Deficiency Hospital (In preparation).
- Johnston, A. W., and Handmaker, S. D. (1960). Primary Amentia and Micro-orchidism associated with an XXXY Sex Chromosome Constitution. *Lancet*, **2**, 184.
- and Weinberg, A. N. (1960). The Chromosome Complement in True Hermaphroditism. *Ibid.*, **2**, 126.
- Ford, C. E., and Hamerton, J. L. (1956). The Chromosomes of Man. *Nature*, **178**, 1020.
- Fraser, J. H., Campbell, J., MacGillivray, R. C., Boyd, E., and Lennox, B. (1960). The XXX Syndrome: Frequency among Mental Defectives and Fertility. *Lancet*, **2**, 626.
- Hamerton, J. L., Jagiello, G. M., Kirman, B. H. (1961). Personal communication.
- Jacobs, P. A., Baikie, A. G., Court Brown, W. M., MacGregor, T. N., Maclean, N., and Harnden, D. G. (1959). Evidence for the Existence of the Human "Superfemale." *Lancet*, **2**, 423.
- Harnden, D. G., Court Brown, W. M., and Baikie, A. G. (1960a). Trisomic Condition of a Large Chromosome. *Ibid.*, **2**, 368.
- — Goldstein, J., Close, H. G., MacGregor, T. N., Maclean, N., and Strong, J. A. (1960b). Abnormalities involving the X Chromosome in Women. *Ibid.*, **1**, 1213.
- Kodani, M. (1958). The Supernumerary Chromosome of Man. *Amer. J. hum. Genet.*, **10**, 125.
- Moore, K. L. (1959). Sex Reversal in Newborn Babies. *Lancet*, **1**, 217.
- Noyes, R. W., Hertig, A. T., and Rock, J. (1950). Dating the Endometrial Biopsy. *Fertility and Sterility*, **1**, 3.
- Ohno, S., and Makino, S. (1961). The Single-X Nature of Sex Chromatin in Man. *Lancet*, **1**, 78.
- Osgood, E. E., and Brooke, J. H. (1955). Continuous Tissue Culture of Leukocytes from Human Leukaemic Bloods by Application of Gradient Principles. *Blood*, **10**, 1010.
- Penrose, L. S. (1961). *Recent Advances in Human Genetics*, Table 6.
- Sandberg, A. A., Crosswhite, L. H., and Gordy, E. (1960). Trisomy of a Large Chromosome. *J. Amer. med. Ass.*, **174**, 221.
- Stewart, J. S. S., Mack, W. S., Govan, A. D. T., Ferguson-Smith, M. A., and Lennox, B. (1959). Klinefelter's Syndrome: Clinical and Hormonal Aspects. *Quart. J. Med. N.S.*, **28**, 561.
- and Sanderson, A. R. (1960). Fertility and Oligophrenia in an Apparent Triple-X Female. *Lancet*, **2**, 21.

STUDY OF CHROMOSOMES IN HUMAN LEUKAEMIA BY A DIRECT METHOD

BY

M. A. KINLOUGH, M.B., B.S.

Myers Research Fellow

AND

H. N. ROBSON, M.B., M.R.C.P., F.R.C.P.Ed.
F.R.A.C.P.Mortlock Professor of Medicine, Department of Medicine,
University of Adelaide, South Australia

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

effect on chromosomes, producing well-differentiated metaphase plates (Kinlough and Robson, 1961). The marrow aspirate or peripheral blood leucocyte concentrate is suspended in hypotonic citrate for 10 minutes and then fixed for two hours in acetic-alcohol. Hydrolysis is then carried out in N HCl for six minutes at 60° C. The cells are then resuspended in 45% acetic acid, and, from this, squash preparations stained with acetic-orcein or with Feulgen are made.

Results

Clinical and haematological details of the 16 patients studied are given in Table I. In eight (six male, two female) the disease was acute in type and course, while the remaining eight patients suffered from the chronic variety—four myeloid and four lymphatic in type.

TABLE I.—Clinical and Haematological Data in 16 Cases of Leukaemia

Case No.	Sex	Age (Yr.)	Type of Leukaemia	Estimated Duration of Disease (Months)	Date of Chromosome Study	Peripheral W.B.C./c.mm. (% Main Cell)	Anti-leukaemic Treatment before Study
1	M	79	Subacute myelo-monocytic Stem-cell	18	25/5/60	30,000 (Mon. 70%)	None
2	F	42	"	1	2/8/60	14,000 (Bl. 90%)	"
3	M	52	Acute myeloid	3	2/9/60	8,000 (Bl. 5%)	"
4	M	29	"	1	22/9/60	10,000 (Bl. 22%)	6 M-P
5	M	32	"	3	3/3/61	146,000 (Bl. 98%)	None
6	M	34	"	1½	12/3/61	86,000 (Bl. 100%)	"
7	F	61	"	3	15/3/61	39,000 (Bl. 90%)	"
8	M	18	"	1	15/3/61	20,000 (Bl. 50%)	"
9	M	40	Chronic myeloid	18	22/9/60	110,000 (P. 72%)	None
10	M	61	"	24	23/11/60	42,000 (P. 60%)	"
11	F	76	"	6	7/2/61	210,000 (P. 49%)	"
12	M	45	"	9	19/5/61	28,000 (P. 68%)	Busulphan
13	F	72	Chronic lymphatic	24	17/5/60	200,000 (L. 80%)	None
14	M	55	"	1 (?)	19/9/60	36,000 (L. 82%)	"
15	M	59	"	24	22/9/60	430,000 (L. 99%)	"
16	F	50	"	24	1/10/60	9,400 (L. 30%)	DXR Chlorambucil

Bl.=myeloblast; P.=polymorph leucocyte; Mon.=monocyte. 6 M-P=6-mercaptopurine; DXR=radiotherapy.

Acute Leukaemia (Cases 1 to 8)

The results of chromosome study in these cases are summarized in Table II. Here it may be seen that six (Nos. 1, 2, 3, 4, 6, and 8) of the eight cases studied showed no significant variation from the normal diploid number of 46. Detailed karyotype study in these cases revealed no constant structural abnormality. Two cases, however, were abnormal (Nos. 5 and 7). In both there was a predominant chromosome number of 47, such cells making up 77% of the total scored in Case 5 and

TABLE II.—Chromosome Counts in Eight Cases of Acute Leukaemia

Case No.	41	42	43	44	45	46	47	48	>48	Total
1	1	1	1	1	5	48	2	3	1	63
2	1	2	—	4	3	18	—	1	—	30
3	—	1	4	4	1	24	3	—	—	37
4	—	2	—	3	1	19	—	—	—	25
5	—	2	—	3	2	11	79	6	—	103
6	1	3	2	2	4	113	7	1	—	133
7	—	—	—	—	5	24	6	—	—	35
8	—	2	—	1	3	40	—	—	—	46

69% in Case 7. Detailed examination of these cells revealed a constant abnormality in karyotype. The karyotypes of the two cases are shown in Figs. 1 and 2 respectively.

The extra chromosome (marked "A" in Figs. 1 and 2) was very similar in both cases. It was of medium size, of the order of the pairs 13–15 of the Denver classification. This extra chromosome, in the cells of both cases, stained densely and showed little arm repulsion. Both patients whose cells showed this abnormality suffered from acute myeloid leukaemia, which had been present for a relatively long period

FIG. 1.—Karyotype of Case 5 (acute myeloid leukaemia) showing chromosome complement of 47. The additional chromosome is marked "A."

FIG. 2.—Karyotype of Case 7 (acute myeloid leukaemia). The additional chromosome is again marked "A."

(three months) when examined. Neither patient had received any treatment prior to chromosome examination (Table I).

Chronic Leukaemia (Cases 9 to 16)

The findings in these cases are summarized in Table III. Examination by the direct technique was more tedious because of the sparser number of mitotic figures. In most cases, however, adequate numbers of suitable cells were found. No numerical abnormality was found in any of these chronic cases; all in fact showed a predominant chromosome count of 46.

TABLE III.—Chromosome Counts in Eight Cases of Chronic Leukaemia

Case No.	41	42	43	44	45	46	47	48	>48	Total
9	—	—	—	3	1	21	—	—	—	25
10	2	3	—	—	1	52	—	—	—	58
11	—	—	—	2	1	40	—	—	—	43*
12	—	—	2	—	—	50	2	2	—	56†
13	—	—	—	—	—	10	—	—	—	10
14	—	—	1	1	—	34	—	—	—	39
15	—	—	1	2	2	44	1	—	—	50
16	—	2	3	1	4	50	1	—	—	61

* 39 of these cells contained the Ph¹ chromosome. † 51 of these cells contained the Ph¹ chromosome.

In two of the cases of chronic myeloid leukaemia, Cases 11 and 12, the "Philadelphia chromosome" was found in over 90% of cells examined in detail. This is illustrated in Fig.

FIG. 3.—A, Chromosome pairs 21 and 22 in a normal subject; B, from Case 11; C, from Case 12, showing the Ph¹ chromosome in chronic myeloid leukaemia.

3. The other two cases of chronic myeloid leukaemia (Cases 9 and 10) were examined at an early stage of this investigation, and the quality of preparations obtained at that time does not permit a decision on whether this abnormality was definitely present or not.

Discussion

As mentioned earlier, the majority of studies to date have reported no abnormality of chromosome number in chronic leukaemia. In the present investigation, all of the eight cases of chronic leukaemia had a diploid number of 46, which accords with the reports of Baikie *et al.* (1959, 1960b, 1961c), Nowell and Hungerford (1960b), Bayreuther (1960), and Tough *et al.* (1961).

The presence of a structural chromosome abnormality in cases of chronic myeloid leukaemia, however, has been confirmed in this study. The abnormally small autosome 21 (Denver)—the Ph¹ chromosome—was present in the great majority of the cells studied in Cases 11 and 12 of the present series. It is of some interest that in these two cases the Ph¹ chromosome was found in higher incidence than that recorded by Tough *et al.* (1961), who found it in 98 out of 132 cells from untreated patients (74%) and in 178 out of 283 cells (63%) from treated patients. It is believed that the higher incidence found here may be due to the fact that the direct method of preparation displays a higher proportion of metaphases from leukaemic cells than does a culture technique. It is also of some interest that one of our cases (No. 12) had had previous treatment with busulphan, which did not seem to have reduced the incidence of the Ph¹ chromosome. This contrasts

with the effects of radiation, which, according to Tough *et al.* (1961), reduced the proportion of cells containing the Ph¹ chromosome. It is not possible to offer comment of any weight on the intriguing suggestion of the Edinburgh workers (Tough *et al.*, 1961) of a relationship between the Ph¹ chromosome and the trisomy of pair 21 in mongolism. The Ph¹ chromosome was not found in any of the acute cases in the present series. Leukaemia in mongolism is usually acute, and the Edinburgh group themselves found only the expected trisomy in five cases of acute leukaemia in mongols.

The position as regards chromosome patterns in acute leukaemia is confused. It has been suggested (Lancet, 1961) that there is no specific pattern and that changes which have been observed are simply the irregularities to be expected in malignant tumours. This is certainly plausible, and derives support from situations such as the one described by Tough *et al.* (1961) where their Case 17, in acute transformation of chronic myeloid leukaemia, was found to have developed a hyperdiploid cell line with 49 chromosomes. (It may be noted that this patient had previously had radiotherapy.)

If chromosomal abnormality was simply an expression of the degree of malignancy the incidence and variety of such abnormality might be expected to correlate roughly with the acuteness of the leukaemia. No such correlation is evident at the present time. So far, studies of some 26 cases of acute leukaemia have been reported by various workers. Of these, normal findings have been reported in 13 cases, and a variety of numerical and structural changes in 10. From the information available it does not appear that the abnormal cases were more acute, either clinically or haematologically, than were those found to be normal. In the present series the two cases with abnormal chromosome complements were distinguished by the fact that the course of the disease was rather less acute than in other cases of the group in which the chromosome number was normal. It is also somewhat surprising to find that both cases had the same abnormal number and that the extra chromosome was so similar in the two cases. Although both cases were of the acute myeloid type, one was a male aged 32 years and the other was a female aged 61. Neither had had any antileukaemic treatment. Similar features of age, sex, and absence of previous treatment were observed in other cases of the series in which the findings were normal. Unfortunately, attempts to culture skin from the two abnormal cases were not successful. However, both patients were normal in physique, development, and sexual function, and an inherent trisomic state seems improbable.

For the reasons stated above we are reluctant to accept entirely the suggestion that these findings in acute leukaemia are merely expressions of the disorder of neoplasia. The remarkably different findings of different groups of workers are perhaps reflected in the findings in this series, where six cases were unequivocally normal and two cases impressively abnormal. Just as the chromosomal situation in chronic myeloid leukaemia appears to be different from that in chronic lymphatic leukaemia, we are tempted to speculate on whether there may be similar differences in different groups of acute leukaemias. Despite the notorious difficulty in identifying the haematological type in acute cases, it would seem desirable to attempt to do this in association with further chromosomal studies.

The development of the direct method for the study of the chromosomes of human leukaemic cells was

prompted by the thought that in leukaemia the cells of the bone-marrow are in a state of frequent division. Therefore it should be feasible to take advantage of this situation in order to avoid the possibility of non-representational development in the artificial conditions of *in vitro* culture. A similar approach was indicated by Sandberg *et al.* (1960) and a similar technique described by Bottura and Ferrari (1960).

The method employed in the present study has proved to be simple and effective, especially in cases of acute leukaemia. As might be expected, the ease with which an adequate number of suitable metaphases can be found is proportional to the degree of marrow activity, as judged on haematological grounds.

A number of authors (Baikie *et al.*, 1959; Ford and Mole, 1959; Nowell and Hungerford, 1960a) have discussed the question of whether the results of culture, even short-term, are representational. The last-named authors, for example, concluded that the relatively long period of colchicine treatment employed by them was responsible for the production of hypodiploid forms in preparations from both normal and leukaemic subjects. These problems of interpretation of findings after culture are avoided by a direct method of preparation. When this is used it may be concluded with some certainty that the findings represent the situation in the cells of the marrow or of the blood at the moment of sampling.

Summary

A method is described which permits the direct study, without culture, of the chromosomes in cells from bone-marrow or from blood.

The study, by this method, of chromosome patterns in 16 cases of leukaemia is described.

The normal diploid number was found in all of the eight cases of chronic leukaemia studied. The abnormally small Ph¹ chromosome was definitely present in two of the four cases of chronic myeloid leukaemia. No abnormality was found in four cases of chronic lymphatic leukaemia.

In six of the eight cases of acute leukaemia studied the chromosomes were normal in number and in structure.

A predominant chromosome complement of 47 was found in two cases of acute myeloid leukaemia. The extra chromosome was of similar character in each case.

We wish to thank Dr. D. L. Hayman for valuable help and advice. The photographs were prepared by Mr. W. Nolan.

REFERENCES

- Baikie, A. G., Court Brown, W. M., Jacobs, P. A., and Milne, J. S. (1959). *Lancet*, **2**, 425.
 ——— Buckton, K. E., Harnden, D. G., Jacobs, P. A., and Tough, I. M. (1960a). *Nature (Lond.)*, **188**, 1165.
 ——— and Jacobs, P. A. (1960b). *Lancet*, **1**, 168.
 ——— (1960c). *Ibid.*, **1**, 280.
 Bayreuther, K. (1960). *Nature (Lond.)*, **186**, 6.
 Bottura, C., and Ferrari, I. (1960). *Ibid.*, **186**, 6.
 Ford, C. E., Jacobs, P. A., and Lajtha, L. G. (1958). *Ibid.*, **181**, 1565.
 ——— and Mole, R. H. (1959). *Lancet*, **2**, 732.
 Kinlough, M. A., and Robson, H. N. (1961). *Nature (Lond.)*. In press.
 ——— and Hayman, D. L. (1961). *Ibid.*, **189**, 420.
Lancet, 1961, **1**, 433.
 Nowell, P. C., and Hungerford, D. A. (1960a). *J. Nat. Cancer Inst.*, **25**, 85.
 ——— (1960b). *Science*, **132**, 1497.
 ——— and Brooks, C. D. (1958). *Proc. Amer. Ass. Cancer Res.*, **2**, 331.
 Sandberg, A. A., Keopf, G. F., Grosswhite, L. H., and Hauschka, T. S. (1960). *Amer. J. hum. Genet.*, **12**, 231.
 Tough, I. M., Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., King, M. J., and McBride, J. A. (1961). *Lancet*, **1**, 411.

CHROMOSOME DAMAGE DURING NITROGEN MUSTARD THERAPY*

A CASE REPORT

BY

P. E. CONEN, M.B., B.S., M.R.C.P.

AND

G. S. LANSKY, M.D.

From the Research Institute and the Department of Pathology, the Hospital for Sick Children, Toronto, and the Department of Surgery, Toronto General Hospital, Toronto

A great quantity of literature has been accumulated on the effects of radiation and radiomimetic substances upon cells. Much of this work has been done on mammalian tissues both *in vivo* and in tissue culture. Some of these damaging effects can be seen by examination of these cells during their division. Abnormal mitoses are common, with an unusual stickiness of the chromosomes and frequent anaphase-lagging. Structural changes may be seen in the chromosomes as either chromatid or chromosome deletions, the production of fragmented chromosomes, and the production of abnormal-shaped chromosomes, particularly ring forms and dicentric. These changes have been demonstrated in human tissues during culture by Puck *et al.* (1957).

After the development of a technique for tissue culture of human peripheral blood, Tough *et al.* (1960) were able to demonstrate abnormal chromosome numbers and patterns in cultures from patients who had received irradiation therapy. Even a single radiation dose of 250 rads produced chromosome changes in a patient receiving therapy over the spine for spondylitis.

We wish to report the case of a patient who showed chromosome abnormalities in the cells cultured from peripheral blood after nitrogen mustard therapy for malignant disease.

Case Report

A 62-year-old man was admitted with fatigue, anorexia, and epigastric pain. He noticed the fatigability and accompanying signs of dry skin and constipation two years before admission, when he first sought medical advice. A hypothyroid condition was diagnosed and 3 gr. (0.2 g.) of thyroid was administered daily. More recently the patient had epigastric pain radiating to the back for a period of three months. He had lost 20 lb. (9 kg.) in weight over a two-year period and weighed 135 lb. (61 kg.) on this admission.

Physical examination showed nothing of note, but a chest radiograph revealed a mass in the left lower lobe. This radiograph was compared with previous chest plates taken two years, one year, and six months previously. The mass was visible on the film taken six months previously although it was then much smaller. Bronchoscopic examination was negative, but bronchoscopic washings showed malignant cells to be present. Tomograms showed the lesion to be approximately 5 cm. in diameter and to involve the subapical segment of the left lower lobe, and there was also hilar enlargement on the left side.

A scalene lymph-node biopsy showed the presence of metastatic carcinoma. Treatment was begun with 12 mg. of nitrogen mustard given intravenously. This was followed one week later by a course of radiotherapy with a tumour dose of 1,200 r per week for three weeks.

*This study was supported by funds from the Ontario Cancer Research Foundation, a Federal Provincial Health Grant, and the Research Institute, Hospital for Sick Children.