It is not easy to see what should be done to prevent symptoms of the kind described in this paper. Faced by a patient with a duodenal ulcer who is known to be a moderate drinker and who has had a number of acute complications of his ulcer, the surgeon must choose whether or not to operate. If he does not do so he may endanger the life of his patient. If he carries out a partial gastrectomy he might convert a reasonably adjusted individual into a chronic alcoholic, incapable of work, a burden to himself and his family alike. A warning about the possible effects of drinking after operation should be given, and if the patient is obviously drinking to excess he should be referred for psychiatric help. Undue preoccupation with dumping symptoms, persistent loss of or failure to regain weight, and repeated requests for potentially habit-forming drugs should all be regarded as warning signals indicating that the patient might be heading for a breakdown or addiction.

In the absence of any clearly defined pattern of behaviour or of personality it is not easy to avoid operating on those who might thereby be exposed to a special risk of breakdown. Those who show psychopathic features or who are prone to resort to drink to relieve their anxieties should be detected before operation so that psychiatric treatment can be started before and continued after operation. By doing this one might hope to avoid some of the unfortunate consequences of partial gastrectomy described in this paper.

Summary

25 patients (17 men and 8 women) were admitted for psychiatric treatment after a partial gastrectomy. In most cases this was done for relief of symptoms of duodenal ulcer. 56% of these cases were admitted for treatment of alcoholism or drug addiction. A review of previous work suggests that there is a special relationship between the operation and subsequent addiction to alcohol and drugs. This special relationship may not hold when psychoneurotic illness follows partial gastrectomy. In such cases the operation probably acts as a non-specific stress in a vulnerable personality.

The implications of these findings are discussed, and it is suggested that the development of certain symptoms post-operatively may indicate the need for psychiatric treatment.

REFERENCES

- Browning, J. S., and Houseworth, J. H. (1953). *Psychosom. Med.*, **15**, 328.
 Delore, P., and Chaupy, A. (1956). *Arch. Mal. Appar. dig.*, **45**, 541.
 Dick, W., Fischer, R., and Sautter, G. (1959). *Dtsch. med. Wschr.*, **84**, 311.
 Hagnell, O., and Wretmark, G. (1957). *J. psychosom. Res.*, **2**, 35.
 Kapp, F. T., Rosenbaum, M., and Romano, J. (1947). *Amer. J. Psychiat.*, **103**, 700.
 Lereboullet, J., Pluvinage, R., and Jungers, P. (1955). *Bull. Soc. med. Hôp. Paris*, **71**, 833.
 Levrat, M., Pasquier, J., and Pasquier, M. J. (1958). *Arch. Mal. Appar. dig.*, **47**, 575.
 Martimor, E., Dereux, J.-F., and Nicholas-Charles, P. (1956). *Presse méd.*, **64**, 1393.
 Navratil, L. (1959). *Münch. med. Wschr.*, **101**, 1088.
 — and Wenger, R. (1955). *Ibid.*, **97**, 1457.
 Poloni, A. (1953). *Minerva med. (Torino)*, **44**, 274.
 Soeder, M. (1957). *Nervenarzt*, **28**, 228.

Copies of *The Prevention of Accidents in Childhood*, a report of a seminar sponsored in 1958 by the Regional Office for Europe of the World Health Organization, are available for persons officially or professionally concerned in this field of study on request to the W.H.O. Regional Office for Europe, Copenhagen, Denmark.

CYTOGENETIC STUDIES IN ACUTE LEUKAEMIA

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Abnormalities of the chromosomes of leukaemic cells in human acute leukaemia have now been the subject of several reports (Ford *et al.*, 1958b; Baikie *et al.*, 1959; Ford, 1960; Sandberg *et al.*, 1960). It would appear that in about half the cases reported some abnormality has been found, including aberrations of chromosome number, morphology, and sometimes both of these. Among the reported cases no two appear to have had an identical abnormality. In this respect, and as regards their frequency of occurrence, the chromosome abnormalities in acute leukaemia may be contrasted with the apparently specific chromosome abnormality which may be found in most cases of chronic myeloid leukaemia in man (Nowell and Hungerford, 1960; Baikie *et al.*, 1960; Tough *et al.*, 1961). It has not yet been possible to correlate any of the clinical or haematological features of acute leukaemia with the varied chromosome abnormalities which have been found. If any such correlations exist it seems probable that they will emerge only after the publication of many more data than are at present available. For this reason we now publish our accumulated cytogenetic results in 22 cases of acute leukaemia in adults, together with the relevant haematological data.

Seven of the 22 patients had a history of significant exposure to ionizing radiation. All seven had, during the preceding 12 years, received radiotherapy for some condition other than leukaemia, involving the irradiation of a large volume of tissue. None of the other patients had any history of radiotherapy. Our knowledge of the occurrence of leukaemia in irradiated individuals indicates that after a single heavy exposure the possibility of leukaemia being diagnosed rises to a maximum between the third and eighth post-exposure years, but may not have returned to normal levels in the twelfth year (Medical Research Council, 1960). In our seven patients with a history of previous radiotherapy there is therefore a high probability that leukaemia was radiation-induced. Inspection of our results suggests that demonstrable chromosome abnormalities may be relatively more frequent among cases of acute leukaemia which may be radiation-induced. Similarly, demonstrable chromosome abnormalities may be relatively more frequent in cases with unusually low white-cell counts. These possible associations can be confirmed or refuted only by the publication of many more data than are now available. With the continued accumulation of results, other associations of possible aetiological or pathogenetic significance may emerge.

In our previous paper on chromosome studies in acute leukaemia we published detailed results in one case and summarized data for four others (Baikie *et al.*, 1959). The complete data for these four cases are now reported together with results in 17 other cases, all of which were

studied before December 1, 1960. The results of similar studies in five cases of acute leukaemia associated with mongolism and three instances of acute transformation of chronic myeloid leukaemia have recently been published (Tough *et al.*, 1961).

Methods and Technical Considerations

The chromosome studies on bone-marrow were carried out by the method of Ford *et al.* (1958b). Peripheral blood leucocytes were cultured by a modification of the technique of Moorhead *et al.* (1960). Initially the chromosomes were spread by squashing between a slide and coverslip, but as this was found to result in a high proportion of broken cells they have more recently been spread by air-drying. The technique for the culture of peripheral blood leucocytes has several important advantages over the method in which bone-marrow is used.

Firstly, the proportion of failures in blood cultures from patients with leukaemia is very much lower than the proportion of failures in bone-marrow cultures from similar patients. Thus in 40 attempts to culture leukaemic marrow there were 25 failures to obtain suitable dividing cells compared with 9 failures in 31 attempts at blood culture. In no case did bone-marrow yield a result where peripheral blood failed to do so.

Secondly, the peripheral blood technique gives chromosome preparations of a very much higher standard than those obtained from bone-marrow cultures. The appearances of the chromosomes are much better in that finer degrees of morphological abnormality may be recognized; and blood preparations generally yield more cells of a quality suitable for chromosome counting.

Lastly, the peripheral blood method has obvious advantages with regard to the ease of obtaining samples, and consequent suitability for serial observations. Nevertheless, in spite of the advantages of peripheral blood culture we feel that it is desirable, at least in acute leukaemia, to attempt chromosome studies on both blood and bone-marrow. This view is confirmed by some of the results we now present.

In spite of the improvement in the number of cells suitable for chromosome counting brought about by the introduction of the peripheral blood method, cultures of leukaemic material yield fewer cells which can be counted than do similar cultures from non-leukaemic subjects. In blood cultures from non-leukaemic subjects there is usually little difficulty in finding at least 50 cells suitable for counting and, if necessary, for analysis. Unfortunately, even this modest number of dividing cells is sometimes not available in preparations of leukaemic blood. This is particularly regrettable, since the determination of the chromosome count distribution of leukaemic material requires the counting of more rather than fewer cells. In fact, it is difficult to set any upper limit to the number of dividing cells it may be desirable to count for the satisfactory determination of the chromosome constitution of leukaemic material. In part this is due to the limitations of the current methods of culture, which are discussed later.

As a consequence of these limitations an abnormal cell line may be grossly under-represented in the final chromosome preparations. If this should occur, examination of a large number of cells may increase the chance of detecting an abnormal cell line such as that found in the blood cultures from Case 21. Even if the culture techniques provide preparations which are

representative of the cell population *in vivo* it may be desirable to count a very large number of cells. For example, in Case 1 the gross deviation from normal of the chromosome count distribution made it desirable to count as many cells as possible for the characterization of the four, or possibly five, cell lines. It is unfortunate that in Case 20, with almost as great diversity of chromosome number, fewer cells could be counted.

Chromosome Count Distribution

At present we must accept the regrettable difference between the number of cells it may be possible to count and the ideal number for the determination of the chromosome count distribution in leukaemia. For the purposes of this report we have been obliged to accept the counting of 30 cells as a minimum number for the determination of chromosome count distribution. Three observations in Table II are based on fewer than this number of cells, but are nevertheless reported in detail because of our earlier brief report of these cases. Several preparations from the new cases failed to yield 30 cells for counting, but in no instance is the finding of an abnormality of chromosome count distribution based on fewer than that number of cells. It should be noted that in four cases (Nos. 8, 9, 20, and 22) fewer than 30 cells could be counted in marrow preparations, while simultaneous observations on peripheral blood yielded more than that number.

The criteria we have adopted for the assessment of the chromosome count distribution in leukaemic blood and bone-marrow depends on comparison with the count distribution in preparations from individuals of apparently normal karyotype. We have taken as normal values an average of 85% modal diploid cells for marrow preparations made by squashing (Court Brown *et al.*, 1960); 78% for blood-culture preparations made by squashing (Jacobs, unpublished observations); and 94% for blood preparations dried in air (Tough *et al.*, 1960). A count distribution is regarded as abnormal if on comparison of the observed and expected proportions of modal cells a marked deficit ($P < 0.01$) of modal (46 chromosome) cells is found. Where the P values are greater than 0.01 the count distribution is regarded as being within the limits of normal. One inadequacy of these criteria lies in their failure to differentiate between the proportions of hypodiploid and hyperdiploid cells among the non-modal cells.

These stringent criteria have been adopted empirically and in spite of the fact that it may be argued that comparisons of the chromosome count distribution of blood and bone-marrow preparations from leukaemic and non-leukaemic individuals are of doubtful validity. Thus it might be said that the greater spread of chromosome count distribution in leukaemic material is due to a greater liability to artifacts consequent upon the increased fragility of the leukaemic cells in some cases of leukaemia. In any event the adoption of these criteria makes it probable that in our results the frequency of abnormality of chromosome count distribution in leukaemic preparations will be underestimated. The adoption of these criteria serves to emphasize the need for basing the assessment of chromosome count distribution, especially in leukaemic material, on as many cells as can reasonably be counted. On the other hand, their uncritical application might lead to a failure to recognize evidence of undoubted chromosome abnormality in some cases.

The assessment of a possible morphological abnormality in chromosome preparations raises fewer

problems. If the same abnormality can be seen in a proportion of the cells studied this is accepted as good reason for regarding the case as one showing undoubted chromosome abnormality. The possibility of these abnormalities having arisen in culture seems improbable, since no similar abnormalities have been found in several hundred cultures from non-leukaemic subjects. Thus it appears reasonable to accept these changes as being directly associated with the leukaemic state.

Almost nothing is known about the possible effects of the treatment of acute leukaemia on the chromosomes of either normal or leukaemic cells. It is known,

however, that agents such as 6-mercaptopurine may produce chromosome abnormalities (Bieseke, 1958). It is likely that any treatment which effectively suppresses an abnormal cell line may influence the results of chromosome studies by altering or masking a chromosome abnormality previously present. Our own observations on the effects of treatment on chromosome abnormalities in acute leukaemia will be the subject of a separate communication. At present we feel that when attempting to score the frequency of chromosome abnormalities in acute leukaemia it is undesirable to count as such any chromosome abnormalities found in

TABLE I.—*Haematological Data—Cases of Acute Leukaemia Without History of Previous Radiotherapy*

Case No.	Age	Date of Diagnosis	Peripheral Blood	Bone-marrow	Leukaemic Cell Differentiation	Treatment (Other than Blood Transfusion)	Date of Chromosome Study	Peripheral Blood	Bone-marrow
1*	56	3/9/58	Hb 4.6, Retic. 0.6, Pl. 78,000, WBC 1,700 (N. 35, Ly. 50, Leuk. 15)	Leukaemic cells predominate	Myelomonocytic	None	3/9/58	As before	As before
2*	64	14/5/59	Hb 9.0, Retic. 4.4, Pl. 95,000, WBC 8,400 (N. 7, Ly. 27, Mon. 6, Bas. 1, Leuk. 59)	Leukaemic cells present	Myeloid	P 15/5/59—	14/5/59 3/8/59	Hb 7.8, Retic. 17, Pl. 73,000, WBC 9,700 (N. 35, Ly. 10, Mon. 8, My. 15, Leuk. 32)	Acute myeloid leukaemia
3*	41	24/11/58	Hb 5.0, WBC 154,000	Acute myeloid leukaemia	"	P—/12/58— M 17/12/58 B ₁₂ 5/1/58 F 20/1/58	5/1/59 19/1/59 10/2/59	Hb 7.9, Retic. 9, Pl. 73,000, WBC 20,000 (N. 31, Ly. 30, Mon. 3, Leuk. 36) Hb 5.1, Retic. 2, Pl. 48,000, WBC 4,800 (N. 28, Ly. 38, Mon. 2, Leuk. 32) Hb 3.5, Retic. 6, Pl. 57,000, WBC 13,900 (N. 2, Ly. 15, My. 1, Leuk. 82)	As before with megaloblastic erythropoiesis Remains megaloblastic Leukaemic appearance unchanged; now normoblastic
4*	48	24/4/59	Hb 12.0, WBC 1,600 (N. 14, Ly. 82, Eos. 2, Mon. 2)	Leukaemic cells predominate	None	P 8/5/59	8/5/59 26/5/59	Hb 9.0, WBC 1,000 (N. 2, Ly. 97, Mon. 1) Hb 9.5, WBC 2,200 (N. 12, Ly. 70, Leuk. 18)	As before " "
5*	70		See Table III						
6	57	17/9/59	Hb 9.2, Pl. 30,000, WBC 40,000 (N. 2, Ly. 16, Eos. 6, Leuk. 76)	Leukaemic blast cells predominate	None	None	17/9/59 2/11/59	As before Hb 6.8, Pl. 20,000, WBC 72,000 (N. 4, Ly. 11, My. 1, Leuk. 84)	As before
7	29	6/10/59	Hb 8.8, Retic. 0, Pl. 50,000, WBC 57,000 (Ly. 12, Mon. 9, Leuk. 79)	" "	Monocytic	"	6/10/59	As before	" "
8	57	—/1/58	Hb 8.3, WBC 3,500 (N. 24, Ly. 64, Mon. 2, My. 2, Leuk. 8)	Bizarre erythroid hyperplasia	Erythroid	P—/2/58 F—/2/58 B ₁₂ —/2/58 all continued	28/1/60	Hb 8.1, WBC 10,800 (N. 21, Ly. 18, Mon. 1, My. 12, Leuk. 48)	Bizarre erythroid hyperplasia with leukaemic blast cells
9	63	1/4/60	Hb 9.0, Retic. 1.0, Pl. 70,000, WBC 30,200 (N. 5, Ly. 10, Leuk. 85)	Leukaemic cells predominate	None	None	1/4/60	As before	As before
10	47	26/5/60	Hb 10.6, Retic. 9, Pl. 10,000, WBC 51,000 (N. 10, Ly. 5, Eos. 5, Mon. 4, My. 8, MMv. 4, PMv. 4, Leuk. 60)	Leukaemic blast cells predominate	Myeloid	"	26/5/60	" "	—
11	55	—/5/60	Hb 6.0, Pl. 75,000, WBC 1,200 (N. 27, Ly. 73)	" "	Lymphatic	"	7/6/60	Hb 11.0, WBC 2,500 (N. 18, Ly. 66, Leuk. 16)	—
12	57	28/6/60	Hb 9.0, Pl. 60,000, WBC 129,000 (N. 7, Ly. 19, My. 1, Leuk. 73)	Leukaemic cells predominate	Myelomonocytic	"	28/6/60	As before	—
13	70	26/10/60	Hb 6.0, Pl. 46,000, WBC 52,000 (N. 6, Ly. 16, Leuk. 78)	Leukaemic blast cells predominate	Lymphatic	"	26/10/60	" "	—
14	61	11/11/60	Hb 7.5, Retic. 6, Pl. 85,000, WBC 148,000 (N. 36, Ly. 2, Mon. 8, My. 3, PMv. 8, Leuk. 43)	Acute myeloid leukaemia	Myeloid	"	11/11/60	" "	As before
15	39	9/8/60	Hb 5.1, Pl. 48,000, WBC 4,000 (N. 36, Ly. 39, My. 3, Leuk. 22)	Myelofibrosis	Myelomonocytic	P—/10/60— —/11/60 A—/11/60 B ₁₂ —/11/60	24/11/60	Hb 7.8, Pl. 23,000, WBC 16,000 (N. 14, Ly. 15, Mon. 10, My. 5, Leuk. 56)	—
16	28	24/11/60	Hb 6.7, Pl. 40,000, WBC 25,300 (N. 32, Ly. 27, My. 5, Leuk. 36)	Acute myeloid leukaemia	Myeloid	None	24/11/60	As before	

* Cases 1–5 previously reported by Baikie *et al.* (1959).

Hb = Haemoglobin (g./100 ml.). Retic. = Reticulocytes (%). Pl. = Platelets (per c.mm.). WBC = White blood cells (per c.mm.). N. = Neutrophil polymorphs (%). Ly. = Lymphocytes (%). Eos. = Eosinophil polymorphs (%). Bas. = Basophil polymorphs (%). Mon. = Monocytes (%). My. = Myelocytes (%). MMv. = Metamyelocytes (%). PMv. = Promyelocytes (%). Leuk. = Leukaemic cells (%). P = Adrenal cortical steroid. A = Corticotrophin. M = 6-Mercaptopurine. B₁₂ = Vitamin B₁₂. F = Folic acid.

treated cases if there is any possibility of these having arisen as a consequence of treatment. Nor can the possibility be excluded in any treated case that an abnormality of chromosome count distribution or morphology, although originally present, may no longer be demonstrable.

The haematological methods used were those of Dacie (1956). In addition, buffy-coat preparations of defibrinated blood were examined, particularly in cases with low peripheral white-cell counts. Whenever possible bone-marrow was examined in both film and squash preparations. It is our belief that, on the whole, attempts at cell-type diagnosis in acute leukaemia are pursued much further than is justified by the reliability of the criteria for distinguishing the various cell types. Nevertheless, in these studies we have attempted to distinguish any possible differentiation of the leukaemic cells in the hope that some correlation of cell-type with chromosome changes might be discerned. The decision concerning cell-type differentiation of the leukaemic cells is based on appearances in preparations stained with May-Grünwald-Giemsa. Account is taken of any evidence of cell-type differentiation either in blood or in bone-marrow at any stage of the disease for which material may be available to us. The inherent observer error in such decisions is greater than is generally recognized (Witts, 1960). We have satisfied ourselves of the magnitude of this error by repeated examinations in which the source of the preparations was unknown to the observers.

Results

For cases without history of radiation exposure the relevant haematological data and the cytogenetic data are given in Tables I and II respectively; and for cases with a history of previous radiation in Tables III and IV respectively. The results of chromosome studies in Cases 1-5 have been reported previously (Baikie *et al.*,

1959); in detail for Case 1, and in summary for Cases 2-5. The full data for Cases 2-5 are reported here, and in two of these the observations have been reassessed in the light of our greater experience. The data for Case 5 now appear in Tables III and IV along with the data for the other cases with a history of radiation exposure.

It will be seen that in 11 patients some abnormality of chromosome constitution was found in the blood or bone-marrow. The abnormalities found in Cases 2, 5, and 8 are not scored as such, either because the observations fail to satisfy our requirements or because possible effects of treatment cannot be excluded. In Case 2 both the cultures yielded an inadequate number of cells suitable for counting. Furthermore, it is possible that in the less unsatisfactory preparations of August 3, 1959, the excess of non-modal cells might be attributable to the effects of treatment. This reservation must also apply to the finding of an excess of non-modal cells in the preparations from the bone-marrow cultures in Case 5 and the blood cultures in Case 8. On the other hand, we have accepted Case 17 as showing definite chromosome abnormality. In this case there was a gross excess of polyploid cells, among which multiples of the haploid number up to sixteenfold were present. In addition, the modal diploid number was shifted to 42, with counts suggesting the presence of at least three cell lines. It seems unlikely that treatment with 6-mercaptopurine for 72 hours could have produced these gross changes in the chromosome count distribution. On the other hand, it might appear not improbable that the excess of polyploid cells was due to the effects of treatment. This possibility is, however, rendered highly unlikely by our knowledge of the appearances of haematological preparations of the bone-marrow made before treatment and at the time the sample was taken for cytogenetic study. In both these bone-marrow smears there were many bizarre multinucleated red-cell

TABLE II.—Cytogenetic Data—Cases of Acute Leukaemia Without History of Previous Radiotherapy

Case No.	Date	Whether Treated	Material	Chromosome Counts													No. of Cells with 46 Chromosomes Expected	P†	Remarks	
				Total	<43	43	44	45	46	47	48	49	>49	Polyploid	Endo-redup.					
1	3/9/58	Untreated	Marrow	184	2	—	3	2	24	45	75	27	6	—	—	156.4	<0.001	Fully reported by Baikie <i>et al.</i> (1959)		
2	14/5/59	Treated	"	12	—	2	—	1	3	3	2	1	—	6	—	10.2	<0.001	No analysis possible*		
	3/8/59			24	—	2	—	2	12	8	—	—	—	2	—	20.4	<0.001	" " "		
3	5/1/59	"	"	64	—	1	3	2	55	3	—	—	—	2	—	54.4	—	No abnormality seen*		
	19/1/59			54	1	—	1	3	48	1	—	—	3	—	45.9	—	" " "			
	10/2/59			61	1	—	1	2	55	1	—	1	—	1	—	51.9	—	" " "		
4	8/5/59	Untreated	"	42	—	—	2	3	36	—	1	—	—	—	—	35.7	—	Constant abnormality, possibly due to translocation or duplication		
	26/3/59	Treated	"	7	—	1	—	—	6	—	—	—	—	—	—	5.9	—			
5											See Table IV									
6	17/9/59	Untreated	Marrow	26	—	—	1	3	22	—	—	—	—	—	—	22.1	0.9 <P> 0.95	No abnormality seen		
	2/11/59	"	"	21	—	2	1	1	16	1	—	—	—	—	—	17.8	0.02 <P> 0.05	" " "		
7	6/10/59	"	"	67	—	—	2	2	41	18	3	1	—	—	—	56.9	<0.001	Excess hyperdiploid cells. In most cells analysed this is due to extra small metacentric chromosome		
8	28/1/60	Treated	Blood	48	—	2	10	13	18	3	1	1	—	—	—	37.4	<0.001	Excess hypodiploid cells. No consistent abnormality		
	28/1/60	"	Marrow	26	4	2	2	2	16	—	—	—	—	—	—	22.1	<0.001			
9	1/4/60	Untreated	Blood	30	1	2	2	3	18	2	2	—	—	2	—	23.4	0.01 <P> 0.02	No analysis possible		
	1/4/60	"	Marrow	29	—	2	1	4	22	—	—	—	—	1	—	24.6	0.1 <P> 0.2	" " "		
10	26/5/60	"	Blood	30	—	—	1	1	28	—	—	—	—	—	—	23.4	—	No abnormality seen		
11	7/6/60	"	"	33	—	1	1	2	29	—	—	—	—	—	—	25.7	—	" " "		
12	28/6/60	"	Marrow	36	2	—	—	2	32	—	—	—	—	—	—	33.8	0.2 <P> 0.3	" " "		
	28/6/60			31	—	—	—	3	27	1	—	—	—	—	—	26.3	—	" " "		
13	26/10/60	"	Blood	40	1	—	1	2	36	—	—	—	—	—	—	37.6	0.2 <P> 0.3	" " "		
14	11/11/60	"	"	50	—	—	—	—	48	2	—	—	—	—	—	47	—	" " "		
15	24/11/60	Treated	"	30	—	—	—	2	28	—	—	—	—	—	—	28.2	0.8 <P> 0.9	" " "		
16	24/11/60	Untreated	"	30	—	1	—	1	28	—	—	—	—	—	—	28.2	0.8 <P> 0.9	" " "		

* Amended from previous report (Baikie *et al.*, 1959).

† χ^2 test. Here and in Table IV the test has been used when the observed number of cells with 46 chromosomes is less than the expected number. Expected numbers: marrow, 85%; blood (squash preparations), 78%; blood (air-dried preparations), 94%.

precursors which may reasonably be equated with the polyploid cells found in the chromosome preparations. These cells were quite as numerous before treatment as at the time of the chromosome studies.

In five cases (Nos. 1, 7, 17, 19, and 20) the chromosome abnormalities included a definite deviation from normal in the chromosome count distribution in accordance with the criteria adopted. The findings in Case 1 suggested the presence of as many as four cell lines with chromosome numbers of 46–49. The normal modal number of 46 was unchanged in Case 7, but there was an excess of hyperdiploid cells with a chromosome count of 47. In contrast, the alteration of modal number was to hypodiploid values in the three previously irradiated patients whose alteration in chromosome count distribution satisfied our criteria (Cases 17, 19, and 20). It is of interest to note that the only other satisfactory demonstration of a hypodiploid chromosome number in leukaemia was also in a patient who developed acute leukaemia some time after radiotherapy. This other case, reported by Ford *et al.* (1958b), had received a total dose of 34 mc of radio-phosphorus for polycythaemia vera.

Where an alteration in chromosome number occurs in leukaemia it may be impossible to decide that particular chromosomes are consistently missing, or present in excess of the normal complement. This difficulty may be due to the presence of several stem-lines, as well as to true variation from cell to cell in respect of the additional or missing chromosomes. Furthermore, attempts to identify these additional or missing chromosomes may be complicated by the concomitant occurrence of morphological abnormalities and chromosome fragments, as in Cases 1, 19, and 20.

In spite of these difficulties it may sometimes be possible to characterize additional chromosomes which are consistently present. For instance, in Case 7, those cells with 47 chromosomes which could be analysed appeared to have an additional small autosome, probably in the group 19–20 (Denver classification). Reference may also be made to our findings (Tough *et al.*, 1961) in a patient with chronic myeloid leukaemia in the phase of acute leukaemic transformation. In this case there were present two distinct cell lines, one with 46 and one with 49 chromosomes. The hyperdiploid line was found to have a constant karyotype; each countable cell carried the Ph¹ chromosome associated with chronic myeloid leukaemia; and the three additional chromosomes comprised a medium submetacentric, a small metacentric, and a small acrocentric chromosome.

Besides the five cases with definite alteration of chromosome count distribution two others (Cases 4 and 21) had morphological abnormalities. In Case 4 an unusually large acrocentric chromosome was seen, possibly the result of a translocation or a duplication. In Case 21, of 52 diploid cells in preparations from peripheral blood leucocytes 6 cells had an abnormal large acrocentric chromosome which was probably due to either a translocation or a pericentric inversion involving one of the chromosomes in the medium-size range (see Fig.). This abnormality was not demonstrable in a marrow preparation.

The results in Case 22 are of particular interest and serve to indicate one of the limitations of the criteria adopted for the assessment of chromosome abnormality. Satisfactory preparations from a blood culture showed a normal count distribution and no morphological abnormality. Only limited success was obtained in the

TABLE III.—Haematological Data—Cases of Acute Leukaemia After Radiotherapy

Case No.	Age	Primary Disease	When Irradiated	Date of Diagnosis of Leukaemia	Peripheral Blood*	Bone-marrow	Leukaemic Cell Differentiation	Treatment* for Leukaemia (Other than Blood Transfusion)	Date of Chromosome Study	Peripheral Blood*	Bone-marrow
5†	70	Poly-cythaemia vera	1945–57	1/9/58	Hb 6.5, Retic. 7, Pl. 20,000, WBC 400 (N. 18, Ly. 44, Mon. 4, Eos. 1, Leuk. 33)	Leukaemic blast cells and megaloblastic erythropoiesis	None	F 1/9/58 – 12/9/58 P 12/9/58	23/9/58	Hb 7.2, Retic. 5, Pl. 60,000, WBC 1,300 (N. 22, Ly. 53, Mon. 1, Eos. 2, Leuk. 22)	Leukaemic elements unaltered. Minimal megaloblastic change
17	44	Ankylosing spondylitis	1954–5	9/6/59	Hb 6.1, Pl. 65,000 WBC 3,400 (N. 34, Ly. 55, Mon. 1, Eos. 6, Leuk. 4)	Leukaemic blast cells and bizarre red-cell precursors	Erythroid	M 19/6/59	22/6/59	Hb 10.1, WBC 1,000	As before
18	59	„ „	1954, 1959	26/8/59	Hb 8.0, Pl. 62,000, WBC 18,800 (N. 60, Ly. 13, Mon. 12, Eos. 1, Bas. 1, Leuk. 13)	—	None	M 31/8/59 – 29/9/59 P 29/9/59 M 9/12/59 – 22/3/60 (intermittently)	8/12/59 22/3/60	Hb 12.1, Pl. 120,000, WBC 16,500 (N. 61, Ly. 23, Leuk. 16) Hb 11.4, WBC 56,700 (N. 49, Ly. 6, Leuk. 45)	Leukaemic blast cells present Unchanged
19	65	Poly-cythaemia vera	1953–9	6/5/60	Hb 8.6, Pl. 99,000, WBC 1,600 (N. 47, Ly. 29, Eos. 3, My. 1, Leuk. 20)	Leukaemic blast cells present	„	None	6/5/60	As before	As before
20	56	Metropathia haemorrhagica	1954	9/6/60	Hb 7.1, WBC 750, (N. 12, Ly. 58, Leuk. 30)	Leukaemic cells present	„	„	9/6/60	„ „	„ „
21	75	Osteitis deformans	1954–8‡	27/10/60	Hb 10.1, Pl. 27,000, WBC 11,900 (N. 53, Ly. 9, Leuk. 38)	Myeloid preponderance with leukaemic blast cells	Myelomonocytic	„	27/10/60	„ „	„ „
22	60	Menorrhagia	1949	11/11/60	Hb 11.7, Pl. 112,000, WBC 23,800 (N. 72, Ly. 11, Leuk. 17). Smear cells 150/200. WBC	Leukaemic blast cells present	Myeloid	„	11/11/60	„ „	„ „

* Abbreviations as in Table I. † Previously reported by Baikié *et al.* (1959). ‡ Phenylbutazone, 1957–9.

TABLE IV.—Cytogenetic Data—Cases of Acute Leukaemia After Radiotherapy

Case No.	Date	Acute Leukaemia Treated or Untreated	Material	Chromosome Counts																P	Remarks
				Total	<40	40	41	42	43	44	45	46	47	48	>48	Polyploid	Endo-Redup.	No. of Cells with 46 Chromosomes Expected			
5	23/9/58	Treated	Marrow	30	—	1	—	2	1	1	5	19	1	—	—	—	—	25.5	<0.001	Excess hypodiploid cells. No analysis possible	
17	22/6/59	„	„	35	2	2	6	13	6	2	1	3	—	—	—	106	—	29.7	<0.001	Great excess polyploid and hypodiploid cells	
18	8/12/59	„	„	55	—	—	—	—	1	—	1	44	6	3	—	1	—	46.7	0.2<P>0.3	Possible excess hyperdiploid cells	
	22/3/60	„	Blood	21	—	—	—	—	1	2	3	13	2	—	—	—	9	16.4	0.05<P>0.1	Large number endoreduplications. Possible translocation	
19	6/5/60	Untreated	„	72	7	8	7	10	11	11	8	9	1	—	—	—	—	56.2	<0.001	58 cells showing structural abnormalities, including rings and fragments	
20	9/6/60	„	„	45	—	—	4	9	18	4	2	6	2	—	—	—	—	35.1	<0.001	Fragments in 20 cells—all hypodiploid	
	9/6/60	„	Marrow	17	—	—	—	—	1	2	—	14	—	—	—	—	—	14.5	0.7<P>0.8	No analysis possible	
21	27/10/60	„	Blood	63	—	—	—	—	1	1	3	58	—	—	—	—	—	59.2	0.8<P>0.9	6 cells showing abnormally large acrocentric chromosome. No other abnormality seen	
	27/10/60	„	Marrow	41	—	—	—	—	—	—	—	41	—	—	—	—	—	34.9	—	No abnormality seen	
22	11/11/60	„	Blood	36	—	—	—	—	—	—	1	34	1	—	—	—	—	33.4	—	—	
	11/11/60	„	Marrow	7	—	—	—	—	—	—	—	1	—	2	4	—	—	6.0	<0.001	3 cells—51 + fragment, all with 5 extra in medium-size range (7-12). 2 other cells had similar fragment

Cell with 46 chromosomes from Case 21 showing an abnormal large acrocentric chromosome.

attempt to culture marrow so that no more than seven cells were suitable for counting and analysis. Of these seven cells, three were found to have 51 chromosomes and a chromosome fragment, and a similar fragment was present in two other cells. Even in so small a number of cells the constancy of these findings must indicate the existence of at least one cell line of abnormal chromosome constitution. This result is accepted as evidence of a definite chromosome abnormality.

Examination of the chromosomes of skin cells was carried out as described by Harnden (1960) in Cases 1, 19, and 20. In all three cases the chromosome constitution of the skin cells was normal. This finding serves to associate the leukaemic state more certainly with the chromosome abnormalities demonstrated in bone-marrow cells in Case 1 and in peripheral blood leucocytes in Cases 19 and 20.

Chromosome preparations from all our cases of acute leukaemia have been examined for the presence of the abnormal chromosome associated with chronic myeloid leukaemia. In none of these 22 cases was the abnormality found, although it may occur where acute leukaemic transformation occurs in chronic myeloid leukaemia (Tough *et al.*, 1961).

The clinical and haematological features of all 22 cases have been reviewed in a search for features which may be correlated either with the frequency of occurrence of chromosome abnormality or with any particular type of chromosome abnormality. Where post-mortem examination was carried out these findings too have been considered. For these purposes 22 cases is a regrettably small series in a disease so variable in its manifestations. It may be pointed out that in no two cases has the same chromosome abnormality been found. By the strict criteria employed chromosome abnormalities were considered to have been found in 3 of 15 cases without a history of previous radiotherapy and in 5 of 7 cases with such a history. If less strict criteria were employed these would be 5 of 15 cases without a history of radiotherapy and 7 of 7 cases with such a history. Attention has already been drawn to the fact that all three cases with change of chromosome counts to hypodiploid values were in the previously irradiated group. There is no obvious association between the occurrence of chromosome abnormalities and the apparent leukaemic cell-type. All the cases were classified as undifferentiated, myeloid, lymphatic, monocytic, myelomonocytic, or erythroleukaemia. With the exception of the two cases in the lymphatic category, one or more cases with chromosome abnormality occurred in each of these cell-type classes. In contrast with this lack of correlation there may, however, be some association between the occurrence of chromosome abnormalities and a low white-cell count in the peripheral blood. Seven patients had counts of fewer than 5,000 white cells per c.mm. at the time of study. Of these, six are known to have died without the count having risen above 5,000; in the remaining case it did not exceed 10,000 before death. In this group of cases with notable and persistently low white-cell levels, only two cases (Nos. 5 and 11) failed to show undoubted abnormality.

Discussion

These results suggest that in acute leukaemia in adults there may be a relatively higher incidence of

demonstrable chromosome abnormalities in cases with low white-cell levels and in patients with a history of significant radiation exposure. We do not intend to discuss the significance of these possible associations with reference to the problems of the aetiology and pathogenesis of acute leukaemia. It seems likely that the continued study of the chromosomes of leukaemic cells may provide information relevant to these fundamental questions. The present needs, however, are, firstly, for data such as we now report on many more cases of acute leukaemia; and, secondly, for some understanding of the relationship of the results obtained by current methods to the state of affairs *in vivo*. Such understanding may be obtainable either by different methods of chromosome study or by modifications of current methods based on knowledge of the metabolism and life-history of leukaemic cells.

The methods of short-term tissue culture at present in use in chromosome studies were developed for the optimal growth of normal peripheral blood leucocytes and bone-marrow cells. It is not surprising, therefore, that leukaemic material rarely grows as well as material from individuals who do not have leukaemia, and that sometimes it does not grow at all. The leukaemic cells in acute leukaemia probably differ from normal blood and bone-marrow cells in both metabolic requirements and in growth-potential. Furthermore, from one case of acute leukaemia to another the leukaemic cells may vary as widely in metabolic requirements and growth-potential as they do in numbers, morphology, and chromosome constitution. These differences, which presumably determine the success or failure of short-term cultures of leukaemic material, may not depend solely on the intrinsic properties of the individual leukaemic cells. Where normal cells are also present or where, as in Case 1, the leukaemic cell population is not homogeneous, interdependence and competition between the cell lines may be important.

At present there is little information available about which cells actually grow when normal blood and bone-marrow are cultured by the methods used in chromosome studies. Presumably in the case of normal bone-marrow the stem-cells of both the erythropoietic and granulopoietic lines proliferate. In cultures of normal peripheral blood leucocytes both direct observations (Nowell, 1960) and less direct evidence (Bond *et al.*, 1959) suggest that the cells which divide are those generally regarded as monocytes and large lymphocytes. In most leukaemic-marrow samples normal stem-cells must be present in large numbers and may compete successfully with the leukaemic cells in culture. In addition, there are other variable factors which may play a critical part when a tissue-culture method is used. These include differences between normal and leukaemic cells in regard to nutritional requirements and intermitotic times. Sampling errors, too, may be introduced either when the chromosome preparations are made from the cultured cells or, as seems less likely, when the original marrow sample is aspirated.

Presumably the operation of one or more of these factors accounts for our findings in Cases 8, 20, and 21, where there were chromosome abnormalities demonstrable in the peripheral blood cultures but no abnormalities in samples of bone-marrow taken at the same time. Some of these variable factors may also operate in the case of peripheral blood cultures, and it would be unwise to assume that in the peripheral blood

in acute leukaemia normal cells capable of division are entirely replaced by leukaemic cells. Thus in Case 22, in satisfactory preparations from peripheral blood, the cells were of normal chromosome constitution. In contrast, in the chromosome preparations from a much less successful marrow culture there was evidence of the presence of a cell line with undoubted chromosome abnormalities. In this case leukaemic cells were equally evident in smears of blood and bone-marrow, but those in the peripheral blood appeared to be extremely fragile, as indicated by the high proportion of smear cells in technically satisfactory blood films. Whatever interpretation may be preferred for the findings in this last case, it is obviously unwise to assume that in all cases of acute leukaemia the leukaemic cells in blood, bone-marrow, and other tissues are identical, at least as regards viability and growth potential.

Ford and Mole (1959) first pointed out that in cultures of marrow for chromosome studies normal cells may outgrow leukaemic cells and so fallaciously indicate a normal chromosome constitution where the leukaemic cells are in fact abnormal. The differences we have found between blood and bone-marrow appear to confirm the validity of their criticism. Sandberg *et al.* (1960) have tried to exclude the possibility of normal marrow cells outgrowing the leukaemic cells by the use of a method in which bone-marrow samples are fixed immediately after withdrawal. The results which may be obtained by this method have not as yet been compared with the results from cultures of the bone-marrow. The culture method provides technically superior preparations with many more dividing cells and thus makes possible the characterization of the chromosome complement of the stem-line or stem-lines. The results yielded by a more direct method such as that advocated by Sandberg and his co-workers could conceivably reflect a situation *in vivo* which cannot be re-created *in vitro*. The method used by Bottura and Ferrari (1960), in which a colchicine derivative is administered to the patient and chromosome preparations are made without preliminary culture of bone-marrow, may have the same merit.

In view of these admitted limitations of methods depending on short-term culture, it is obvious that, in individual cases of acute leukaemia, the finding of a normal chromosome constitution is less significant than the finding of an abnormality. It is interesting to note that Ford *et al.* (1958a) found chromosome abnormalities in a very high proportion of reticular neoplasms in the mouse, whether they were spontaneous or radiation-induced. In these studies the chromosome preparations were made directly from tumour tissue taken from animals previously given demecolcine ("colcemid"), so that culture was avoided. It may well be that with the methods at present available the most satisfactory chromosome studies in acute leukaemia could be achieved by combining a direct examination of bone-marrow with the culture of both bone-marrow and peripheral blood. In the future, with some knowledge of the nutritional requirements and life-span of leukaemic cells, it may be possible to adjust the conditions of culture from case to case, so that growth *in vitro* reflects more faithfully the position *in vivo*.

Summary

Chromosome studies have yielded satisfactory results in 22 out of 31 cases of acute leukaemia in adults. In eight cases results were obtained from bone-marrow, in

seven cases from peripheral blood leucocytes, and in seven others from both tissues.

It is suggested that an attempt be made to establish criteria of chromosome abnormality applicable to leukaemic material. By the strict criteria adopted, chromosome abnormalities are thought to have been found in eight cases. Of seven patients with a history of significant exposure to radiation, five had undoubted chromosome abnormalities. In contrast, of 15 cases without such a history definite chromosome abnormalities were found in 3. Seven patients had white blood cell counts of fewer than 5,000 per c.mm. and chromosome abnormalities were found in five of them.

These findings suggest that the clinical and haematological features of cases of acute leukaemia should be considered and published with the results of chromosome studies. The possible association of chromosome abnormalities with these other features suggested by our results can only be confirmed or refuted by the accumulation of many more data than are at present available.

The limitations and possible disadvantages are discussed of methods of chromosome study dependent on short-term tissue culture.

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REFERENCES

- Baikie, A. G., Court Brown, W. M., Buckton, K. E., Harnden, D. G., Jacobs, P. A., and Tough, I. M. (1960). *Nature (Lond.)*, **188**, 1165.
 ——— Jacobs, P. A., and Milne, J. S. (1959). *Lancet*, **2**, 425.
 Bieseke, J. J. (1958). *Ann. N.Y. Acad. Sci.*, **71**, 1054.
 Bond, V. P., Flidner, T. M., Cronkite, E. P., Rubini, J. R., Brecher, G., and Schork, P. K. (1959). *Acta haemat. (Basel)*, **21**, 1.
 Bottura, C., and Ferrari, I. (1960). *Nature (Lond.)*, **186**, 904.
 Court Brown, W. M., Jacobs, P. A., and Doll, R. (1960). *Lancet*, **1**, 160.
 Dacie, J. V. (1956). *Practical Haematology*, 2nd ed. Churchill, London.
 Ford, C. E. (1960). *Proc. roy. Soc. Med.*, **53**, 491.
 ——— Hamerton, J. L., and Mole, R. H. (1958a). *J. cell. comp. Physiol.*, **52**, Suppl. 1, p. 235.
 ——— Jacobs, P. A., and Lajtha, L. G. (1958b). *Nature (Lond.)*, **181**, 1565.
 ——— and Mole, R. H. (1959). *Lancet*, **2**, 732.
 Harnden, D. G. (1960). *Brit. J. exp. Path.*, **41**, 31.
 Medical Research Council (1960). The Hazards to Man of Nuclear and Allied Radiations, 2nd Report. H.M.S.O., London.
 Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A. (1960). *Exp. Cell. Res.*, **20**, 613.
 Nowell, P. C. (1960). *Ibid.*, **19**, 267.
 ——— and Hungerford, D. A. (1960). *Science*, **132**, 1497.
 Sandberg, A. A., Koepf, G. F., Crosswhite, L. H., and Hauschka, T. S. (1960). *Amer. J. hum. Genet.*, **12**, 231.
 Tough, I. M., Buckton, K. E., Baikie, A. G., and Court Brown, W. M. (1960). *Lancet*, **2**, 849.
 ——— Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., King, M. J., and McBride, J. A. (1961). *Ibid.*, **1**, 411.
 Witts, L. J. (1960). Paper read to the British Society for Haematology. London, November, 1960.