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.

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CHROMOSOME DAMAGE DURING NITROGEN MUSTARD THERAPY*

A CASE REPORT

BY

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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.

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The patient died seven weeks after the starting of treatment, and at necropsy a bronchogenic carcinoma arising in the left lower lobe, with metastases in both adrenal glands and the para-aortic lymph nodes, was found.

Chromosome Studies

Chromosome preparations were made from peripheral blood samples by a modification of the method described by Moorhead *et al.* (1960). One sample was taken before treatment, and two further samples were taken after nitrogen mustard treatment but before radiation treatment had begun. The effects of nitrogen mustard treatment upon leucocyte chromosomes were investigated by comparison with the control samples before treatment. The findings are summarized in the accompanying

Days After Dose	*Total Cells Counted	Distribution							Chromosome Structural Change		
		<44	44	45	46	47	48	4n	†No. of Cells Examined	No. of Cells Abnormal	% Ab-normal
-6	25			2	23			<1%	50	0	0
+2	None										
+4	76	2	3	3	64	4		<1%	160	7	4.5

* Does not include cells with a visible structural chromosome abnormality.
† Includes "total cells counted."

FIG. 1.—A: Abnormal chromosomes present, including fragments (F) and dicentric (D). B: Partially analysed karyotype.

Table, and showed a normal chromosome number and XY sex-chromosome complement in the cells before treatment was started and a greater variation in chromosome numbers and structural abnormalities of the chromosomes after treatment. The culture taken two days after the nitrogen mustard treatment failed to provide enough cells in mitosis for any worth-while analysis. The few that were present were unsuitable for accurate counting and appeared to be shrivelled. The paucity of mitoses was probably related to the leucopenia usually produced by this treatment. The culture prepared from blood taken four days after the nitrogen mustard provided many mitoses suitable for detailed examination. Abnormal chromosomes were present and included fragments (F) and dicentric (D) (Fig. 1,A).

Fig. 2 shows examples of other chromosomes of unusual shape that were found. Fig. 2,A appears to represent a chromatid deletion of a chromosome in the 13-15 group, although we have on rare occasions seen this phenomenon in cultures from patients where there was no known history of exposure to x rays or radio-mimetic agents. Fig. 2,B appears to represent a fusion of three chromosomes. No ring chromosomes or tracentrics were seen. Analysis of cells showing chromosome damage is very difficult and interpretation uncertain, so that only a partially analysed karyotype is shown in Fig. 1,B.

FIG. 2.—A: Showing ? chromatid deletion. B: ? Chromosome fusion.

The value of chromosome counts in this type of case is difficult to assess. Total counts in cells with structural chromosome changes are sometimes uncertain, since unusual shapes may be formed, causing difficulty in interpretation. Fragments are conventionally counted as a chromosome by most but not all workers. In cultures from cases without radiomimetic damage, the significance of chromosome counts differing from the modal number can be partially tested by analysing them to see if the abnormal count is consistently associated with an abnormal number of chromosomes in one group. In the type of case presented this precaution is not possible, because the radiation would also damage different groups of chromosomes. Very large numbers of cells should be counted before alterations of the modal-cell percentage can be regarded as significant. However, in this laboratory it has been noted that cell breakage during chromosome preparation only rarely produces cells with counts above the modal number, whereas some cells are usually found with counts below this number. But in this case, and in other cases receiving radiomimetic agents, a significant number of counts above the modal number has been observed. The technique of air-drying the cells to obtain flattening, which is so successful with tissues grown directly on glass, greatly reduces the number of broken cells (Rothfels and Siminovitch, 1958). However, it does not seem to give so many good clear metaphase plates as the squashing method, when applied to cultures of blood or marrow.

Tough *et al.* (1960) found a considerable increase in polyploid cells after irradiation. In one patient the incidence of polyploids was normal four days after irradiation, then increased and remained elevated for many days. The second patient showed many polyploid cells the day after treatment, but the incidence fell to normal levels until a second increase in polyploid cells occurred one week later. Boyd *et al.* (1961) reported chromosome aberrations after radioiodine treatment and also noted an increased incidence of tetraploid cells. There was no noticeable increase in tetraploid cells in the culture made four days after giving nitrogen mustard in our case. Further samples at different time intervals might have shown an increased number of tetraploid cells.

Discussion

The increase in percentage of non-modal chromosome counts compared with the pretreatment control counts is related to the radiation-type damage. If only the cells with apparently intact chromosomes are counted this will include only part of the population of cells with chromosome damage. Cells containing structural chromosome abnormalities are easily recognized, and a large number of cells can be examined quickly without chromosome counts. Probably a far larger number of cells show no visible evidence of chromosome damage and repair, and will escape detection. However, a combination of the non-modal chromosome counts and the numbers of cells showing chromosome damage will give a low estimate of radiation damage. The use of examinations for structural abnormalities should be stressed, since cells can be screened by a technician and "suspicious" cells checked by a more experienced worker.

The chief significance of this report is that, if nitrogen mustard can cause chromosome aberrations in leucocytes, abnormalities may also be produced in the germ cells, since the nitrogen mustard is carried to all parts of the body. Some slight control of the distribution

of this agent is possible by using the recently developed regional perfusion techniques. Although the side-effects of this drug are severe for many hours, it is possible that an abnormal zygote could be conceived in the few days following the radiomimetic damage to the germ cells. This would be more likely after treatment of males, since a female with malignant disease would be less likely to conceive or carry a child to term. The problem of the relationship between radiomimetic agents and the later development of leukaemia is of academic interest only because of the prognosis expected in this type of case.

Summary

Chromosome studies were made on blood samples from a patient with malignant disease before and during nitrogen mustard therapy. Structural chromosome abnormalities were demonstrated in these leucocyte cultures. Nitrogen mustard is widely distributed in the body and presumably could cause similar damage to the germ cells. This might provide a mechanism for the formation of an abnormal zygote.

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ATHEROMA OF THE CAROTID AND VERTEBRAL ARTERIAL SYSTEMS

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The importance of disease of the neck arteries in the causation of strokes was first appreciated last century (Todd, 1844; Gull, 1855), and was emphasized by Ramsay Hunt (1914); but, in general, the condition of the intracranial arteries was still held to be the most important factor. The introduction of angiography and the practicability of surgical intervention has tended to reverse this view by focusing increasing attention on the neck arteries. Hutchinson and Yates (1957) stressed the importance of considering the carotid and vertebral systems together, and reported that of 83 patients with cerebral vascular symptoms 40 had marked narrowing of one or more of the neck arteries.

We felt that in order to put this finding into perspective it was important to assess the condition of the neck arteries in a consecutive and unselected series of necropsies. We have studied 93 such patients and report here our findings, with respect to the severity and localization of atheroma, the frequency and site of stenosis, and the presence of occlusion and ulceration.

Methods of Study

The carotid and vertebral systems were removed from every fifth patient over the age of 35 on whom a