10% pyridine. Care was taken to exclude any influence of impurities from the chromatography paper onto which GIF was adsorbed prior to extraction. The results obtained are given in Figure 1. GIF seems to be extracted almost quantitatively into the solvents used. This is in accordance with the observations made with the 1-butanol extractions, where a high affinity to the aqueous phase was found. The 10% acetic acid extract exhibits a very strong inhibition as compared to the controls and the other extracts. This may be due to traces of acetate ion which could not be removed by evacuation over KOH pellets. Acetate ion has been found to inhibit the thymidine incorporation into bone marrow cells at concentrations as low as 10^{-5} M [14]. The presence of acetate ion may result from pH shifts at the final stages of evaporation when phosphate buffer salts (pH 7.4) and acetic acid are present at comparable concentrations, and part of the acetic acid is converted to sodium acetate which is not volatile. These effects are difficult to control and reproduce, which may explain the observed discrepancies between control and experimental values. For this reason, it may be advantageous to avoid acetic acid as a solvent during chromatography of GIF[2].

Solubility of GIF in organic solvents. The solubility of GIF in solvents of decreasing polarity, a) ethanol-acetone (9:1)[15], b) chloroform-methanol (1:1), and c) chloroform is given in Figure 2. From these results it appears that GIF (adsorbed onto cellulose) can be extracted almost quantitatively into organic solvents of not too low polarity. The quantitative recovery of GIF after extraction with a 1:1 mixture of chloroform and methanol, however, makes it possible to extract this inhibitor from tissue homogenates without co-extracting large amounts of accompanying material. A crucial step during the purification of GIF might thus be simplified to a considerable extent.

The preparations of GIF used in these investigations show a rather high inhibitory activity of approximately 70%. Since, with pure GIF, inhibition should be well below 50%, other inhibitory substances seem to be present in the GIF solutions. An erythropoiesis inhibiting factor has been described[16] which elutes in the same region as GIF. The GIF preparation used in these experiments is not cytotoxic, as judged by the method of PACSA[17], which uses a colorimetric assay for cell respiration for the detection of cytotoxicity. Methods for the complete purification of GIF, especially the removal of any other inhibitory substances, will be subject of future research.

Summary. The solubility of granulopoiesis inhibiting factor (GIF) in various aqueous and organic solvents was investigated. GIF is soluble in water, 10% acetic acid, and 10% pyridine. It is not extractable by 1-butanol at low and high pH. A high solubility was found in polar organic solvents (ethanol-acetone 9:1, and chloroform-methanol 1:1), whereas GIF seems to be insoluble in pure chloroform.

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Ring Chromosomes and Leukaemia

Through the application of banding techniques, some types of acute leukaemia have been classified, at least tentatively, according to their chromosome pattern[1], but the relationship between specific aberrations and clinical-haematological course remains obscure in most cases.

Ring chromosomes have been described in a few cases of erythroleukaemia (EL)[2-4] and acute myelogenous leukaemia (AML)[5,6]; their presence probably has unfavourable prognostic significance. We have observed ring chromosomes in 2 out of 100 cytogenetically studied cases of acute leukaemias.

Case I. A 68-years-old farmer was admitted to hospital in December 1973, because of weakness, precordial pains, arthralgias, severe anaemia and hepatosplenomegaly. Blood examination revealed: Hb = 6.5 g%, WBC = 2,100/ml, with lymphocytes 70%, monocytes 17%, neutrophils 13%; platelets 95,000/ml. Bone marrow was hypocellular. The erythroblastic series was dominated by early erythroblasts, with increased nucleo-cytoplasmatic ratio, multinucleation, delicate chromatin network, megaloblastosis and atypical mitoses. A number of 'blasts', monocytoid in appearance, were found. The granulocytic and megakaryocytic series were scanty, but normal. The patient was treated with corticosteroids and androgens. Two subsequent bone marrow biopsies in April and May 1974 revealed a picture closely comparable with that obtained in the first study, with an increase of 'blast' cells. On the basis of cytochemical and cytoenzymatic data, a diagnosis of acute EL was posed. The conditions of the patient rapidly worsened, with increasing anaemia, leucopenia and presence of blasts and erythroblasts in the peripheral blood, and he died in September 1974.

Chromosome studies were performed on direct bone marrow preparations in April and in May 1974. In the first preparation 25 metaphases were analyzed: chromosome number ranged from 34 to 92, with a modal number of 43 in 13 cells. One more rings, the size of a G- or occasionally of an E-group chromosome, were observed in 13 cells (Figure 1). 15 cells were analyzed from the second preparation. Chromosome number ranged from 38 to 83, with a prevalance of mitoses with 40 chromosomes. One or more rings were observed in 14 metaphases.

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Case II. A 76-year-old housewife was hospitalized in June 1974 for anaemia and cerebral ischaemia. At the first blood examination, Hb was 8.6 g%, WBC 7,100/ml, with several atypical 'monocytoid' cells. 2 weeks later, WBC were 12,000/ml, with 'monocytoid' cells 25%, myelocytes 3% and erythroblasts 2%. Cytochemical and cytoenzymatic studies suggested a diagnosis of acute myelo-monocytic leukaemia. Bone marrow investigations were not performed because of the poor clinical condition of the patient. She was treated with corticosteroids and blood transfusions. In September 1974, WBC were 180,000/ml, with 'blast' cells 55%; platelets 90,000/ml. She died on 20 September 1974 of cerebral haemorrhage, when WBC were 280,000/ml.

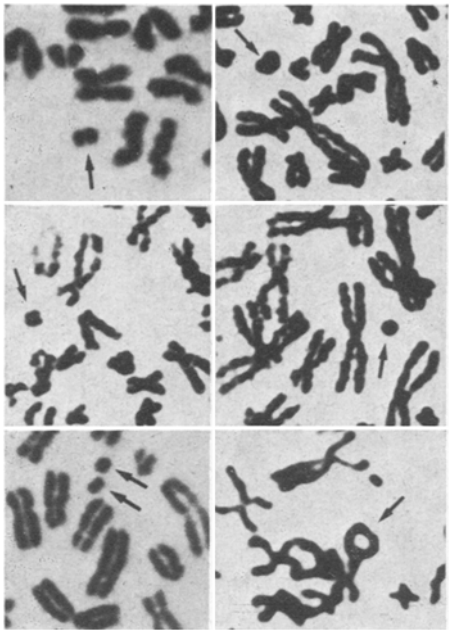

Fig. 1. Case I: partial metaphase plates showing ring chromosomes.

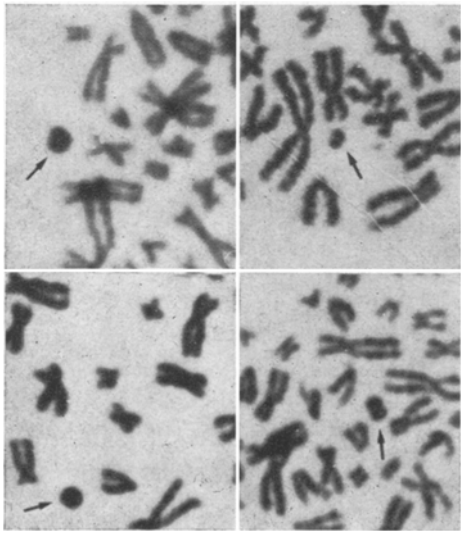

Fig. 2. Case II: partial metaphase plates showing ring chromosomes.

Two direct chromosome preparations were obtained from peripheral blood in September 1974. 22 metaphases were analyzed; chromosome number ranged from 44 to 49 with a mode at 47 chromosomes. Structural rearrangements were observed in almost all groups, together with the constant presence of a ring chromosome, the size of an E-or G-group chromosome (Figure 2).

From available data, some conclusions can be drawn concerning the association between ring chromosomes and leukaemia: 1. The presence of rings in malignant haemotological disorders is rare. 2. 2 out of 3 AML with rings could be in connection with previous antimitotic treatment[5] or exposure to ionizing radiations[6]. 3. Rings have been reported in association with 4 cases of untreated acute EL. Remembering that in EL, as in megaloblastic anaemia, there are signs of impaired DNA and nuclear metabolism[4], and that chromosome breaks are frequent in bone marrow preparations[7], it is conceivable that the preferential association of rings with EL might be secondary to the chromosomal instability. 4. Rings occur in elderly patients (mean age 65 years) and their presence is associated with a short survival (about 4 months). They appear when the malignant process has evolved to a preterminal stage, marking a picture of gross chromosome rearrangements. Since the median survival is significantly shorter in patients with acute leukaemias who never had normal metaphases in their bone marrow during the course of the disease, when compared with subjects with both abnormal and normal metaphases, or those with only normal metaphases[8], the unfavourable prognostic significance of the presence of rings could not be directly related to the rings themselves, but to the chromosome unbalance of which rings are an uncommon epiphenomenon.

Summary. The observation of 2 leukaemic patients with ring chromosomes, and their comparison with a few previously reported cases, suggests a close association between this rare chromosomal abnormality and erythroleukaemia and indicate a poor prognostic significance of this finding.

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