sociated by urea into 4 sub-units: one which does not penetrate the gel, another with low mobility and 2 faster moving subunits. Collagen polymerized in the presence of factor $XIII_a$ is dissociated into 1 sub-unit which does not penetrate the gel, and a series of slow moving units. There are no fast moving sub-units. An identical pattern to that observed in the absence of factor XIII is obtained when factor $XIII_a$ is previously incubated with MIA. When factor $XIII_a$ incubated with GME is used, the results closely resemble those obtained in the absence of factor XIII, although small variations are noted.

3. Contrast phase microscopic examination. As shown in Figure 2, it seems that collagen fibres are assembled in bunches in 3 cases: in the absence of factor $XIII_a$, in the presence of factor $XIII_a$ previously incubated with GME or MIA and in the presence of thrombin (the enzyme necessary for factor XIII activation). In the presence of factor $XIII_a$, on the other hand, the fibres become tangled and never appear in linear pattern.

Discussion. The collagen molecule is made up of 3 chains (2 α_1-chains, and 1 α_2-chain) interlinked by hydrogen bonds and a few covalent links of the Schiff base type[5] According to the previous literature[6] and considering the results of the polyacrylamide gel electrophoresis experiments, collagen polymerized in the absence of factor XIII is dissociated into 3 types of elements after incubation with urea and SDS: γ-elements made up of α-trimers, which does not penetrate the gel; β-elements, made up of α-dimers, with low mobility; and the α_1- and α_2-chains, sub-units of faster mobility. On the other hand, collagen polymerized in the presence of factor $XIII_a$, is dissociated in the same conditions, into only 2 types of sub-units: the first which does not penetrate the gel and the second slow migrating, consisting of α-dimers. No isolated α_1- or α_2-chains are found. The determination of the molecular weight of the different sub-units from their mobility is impossible, because of the fibrillar (and not globular) structure of the 2 chains of the collagen molecule[7]. These results suggest that factor $XIII_a$ brings about the formation of stable bonds between the collagen chains (absence of isolated chains). Furthermore, since factor $XIII_a$ induces a decrease in the quantity of collagen polymerized, these links probably block the sites used for hydrogen bonding which is necessary for polymerization.

The results obtained by phase contrast microscopy seem to support this intermolecular linkage hypothesis. Indeed, collagen polymerized in the absence of factor $XIII_a$ seems to be organized into bunches, whereas, in the presence of factor $XIII_a$ the fibres seem assembled at random.

The formation of stable bonds seems due to factor $XIII_a$. Indeed, the polymerization of collagen (quantity of collagen polymerized, PAG electrophoresis and contrast phase microscopy) is similar – when factor XIII is absent – or when thrombin is added (coagulation proteolytic enzyme used for the activation of factor XIII), or when factor $XIII_a$ is previously incubated with MIA (inhibitor of the factor XIII activity, on fibrin stabilization, by alkylation of its sulfhydril groups[8]) is used. Furthermore, the increase in the quantity of polymerized collagen and the bunching of fibres observed by phase contrast microscopy, when GME is added to factor $XIII_a$, seems to prove that factor $XIII_a$ promotes the formation of transamidation bonds, as in fibrin stabilization (GME is an inhibitor of the transamidation reaction). In this instance, however, the pattern of collagen sub-units in PAG electrophoresis is not identical with the one observed in the absence of factor XIII (the percentage of isolated chains of collagen seems slightly less important), probably because GME is a competitive inhibitor.

To conclude, our results suggest that factor $XIII_a$ brings about the formation, between the collagen molecule constituents, of transamidation bonds (similar to those obtained by the action of factor $XIII_a$ on fibrin) and induces a decrease in the quantity of collagen polymerized, probably by blocking the groups involved in the formation of hydrogen bonds. This action of factor XIII possibly accounts for its importance in normal healing.

Summary. Factor XIII induces a decrease and a modification in the collagen polymerization, probably due to the formation of transamidation bonds. This property has some importance in wound healing.

A. Soria[9], C. Soria and C. Boulard

Centro Hospitalo-Universitaire de Paris, Service d'Hématologie de l'Hôtel-Dieu, laboratoires de Biochimie et d'Hématologie de l'Hôtel Dieu, Place du Parvis Notre-Dame, F–75181 Paris 4 (France), Laboratoires de Biochimie et d'Hématologie, Hôpital Lariboisière, Paris 10 (France), and Institut National Agronomique, Laboratoire de Zoologie, Paris 5 (France), 14 July 1975.

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The Solubility Properties of Granulopoiesis Inhibiting Factor

Granulopoiesis inhibiting factor (GIF) is a postulated humoral regulator of cell production which is released by mature granulocytes, and which, by a negative feedback mechanism, inhibits the production of myeloid cells in the bone marrow. The biological properties of GIF, especially its exclusively specific action on granulopoiesis[1–5], have been interpreted as GIF being the chalone of the granulocytic system. Attempts to purify this substance have so far been only partially successful[1, 2, 6–8]. In this paper, the solubility properties of GIF in a series of different solvents

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Extraction of GIF with 1-butanol

	cpm ^{3}H-TdR/10^6 cells/30 min
Controls	
0.01 *M* Phosphate buffer	6032 ± 150
Untreated GIF-solution	2189 ± 230
Extraction from 0.01 *M* HCl	
Butanol-phase	5878 ± 95
Aqueous phase	1452 ± 35
Extraction from 0.01 *M* ammonia	
Butanol-phase	6133 ± 120
Aqueous phase	1769 ± 41

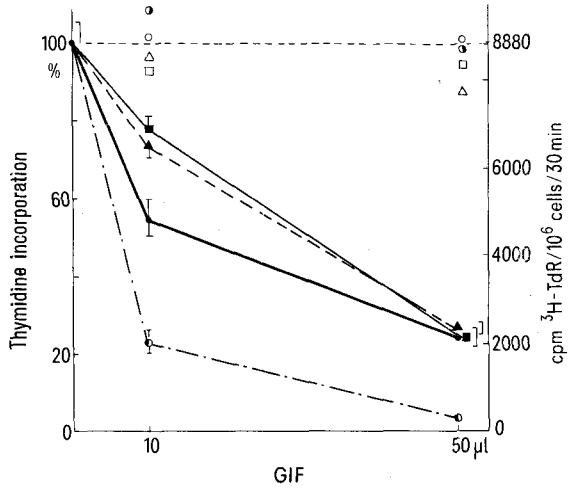

Fig. 1. Extraction of GIF adsorbed to cellulose into aqueous solvents. ●, control (untreated GIF solution); ■, water; ▲, 10% pyridine, ◐, 10% acetic acid. The data points represent the mean of 4 parallel determinations, standard deviations are indicated as bars. Open symbols (○ □ △ ◑) represent the respective buffer controls.

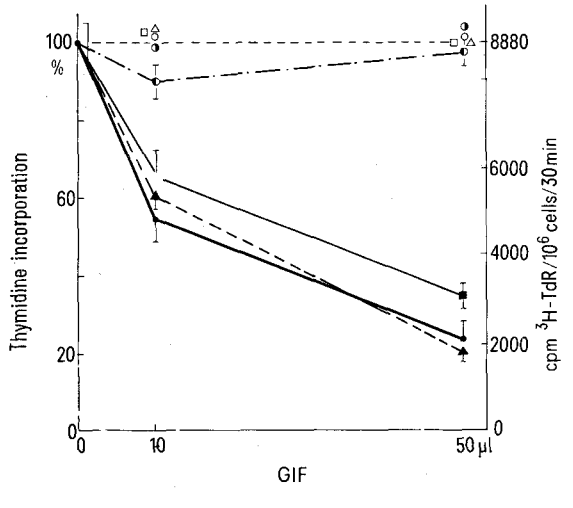

Fig. 2. Extraction of GIF from cellulose into organic solvents. ●, control (untreated GIF solution); ▲, ethanol-acetone (9:1); ■, chloroform-methanol (1:1); ◐, chloroform. The mean of 4 parallel determinations and the standard deviations are given. Open symbols (○ △ □ ◑) represent the respective buffer controls.

have been investigated in order to extract GIF from biological material without simultaneously extracting large amounts of contaminants.

Methods and materials. Preparation and assay of GIF. The preparation of GIF from rat bone marrow conditioned medium and its partial purification by column chromatography on Sephadex G-25 and G-15 have been described elsewhere[2]. The determination of inhibitory activity is based on the measurement of thymidine incorporation into bone marrow cells in vitro. The cultures contained in a final volume of 0.3 ml: 10^6 bone marrow cells, 0.2 ml RPMI-1640-medium, 0.02 ml fetal calf serum, 0.01 ml horse serum, 0.05 ml of the GIF-solution, 30 m*M* morpholino-propane-sulfonic acid (pK = 6,7)[9]. After 90 min at 37 °C, 0.5 µCi ^{3}H-Thymidine (sp. activity 20,000 mCi/mmole) were added and the cultures were terminated at 120 min. The radioactivity in the trichloroacetic acid insoluble material was determined according to SÜSS and VOLM[10].

Extraction with 1-butanol. The GIF solution was made 0.01 *M* in HCl and extracted 4 times with an equal volume of 1-butanol equilibrated with 0.01 *M* HCl. The combined organic layers and the aqueous phase were evaporated to dryness in vacuo over KOH and P_2O_5. The residues were dissolved in water and aliquots were removed for the GIF assay. The residue from the aqueous layer was made 0.01 *M* in ammonia and extracted 4 times with an equal volume of 0.01 *M* NH_3-saturated 1-butanol. The organic and aqueous phases were treated as described and aliquots were used for the GIF-assay.

Solubility of solid GIF in various solvents. The GIF solution, which was essentially a solution in 10 m*M* phosphate buffer, was applied in portions of 200 µl to small strips of Whatman 3 *MM* paper, which were pretreated with the solvents used later for the extraction of GIF. After drying, the strips were immersed in 5 ml of the following solvents: a) water, b) 10% acetic acid, c) 10% pyridine, d) ethanolacetone (9:1, v/v), e) chloroform, f) chloroform-methanol (1:1, v/v). After 5 h at room temperature, the strips were removed and the solvents evaporated in vacuo over KOH and P_2O_5. The residues were dissolved in 0.2 ml 10 m*M* phosphate buffer (or water in the case of aqueous solvents) and the biological activity was determined. 10 m*M* phosphate buffer applied to 3 MM strips and treated in the same way was used as control.

Results and discussion. Butanol-extraction. The effect of aliquots of the extracts on the thymidine incorporation into bone marrow cells showed that the inhibiting activity remained in the water phase, irrespective of the pH of the solution, as is given in the Table. GIF thus seems to be a relatively polar molecule, but the polarity may be independent of the pH of the solution. There is evidence that GIF contains peptide bonds[11]; however these polarity properties demonstrate that it behaves differently from some simple polypeptides (e.g. calcitonin or melittin) which can be extracted into 1-butanol from acidic or basic solutions[12, 13]. The usual ionizable groups of peptides thus seem to have no major effect on the solubility properties of GIF.

Solubility of GIF in aqueous solvents. Dose-response curves were determined for the original GIF solution and for the extracts obtained with water, 10% acetic acid and

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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.

W. R. PAUKOVITS and J. B. PAUKOVITS

Institut für Krebsforschung der Universität, Borschkegasse 8a, A-1090 Wien (Austria), 17 February 1975.

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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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