amounts and with high frequency by the Chilean population. In addition, several secondary amines such as piperidine and pyrrolidine have been found in cigarette smoke[14,15]. Since secondary amines react with nitrite to form nitrosamines in vitro in human gastric juice[11] and in vivo inducing multiple oesophageal tumours in rats[16], nitrite should only be used (as food additive) in the lowest amounts, i.e., the minimum capable of controlling botulism. In 1973 Norway banned nitrite as a flavouring and colouring agent, permitting it only for preventing food poisoning caused by *Clostridium botulinum*.

The correlation presented in this communication is apparently not an artefact nor a causal association but an indirect association, i.e., a factor (nitrate fertilizer) and a disease (gastric cancer) are associated only because both are related to some common underlying condition or process (nitrosamine formation). However, at present we cannot entirely rule out a carcinogenic or co-carcinogenic effect of dietary nitrate per se on the human stomach.

Since ZALDÍVAR holds the hypothesis that oesophageal and gastric carcinomata in man have a multiple factor aetiology, and started in 1966 a new multidisciplinary, multifactorial approach measuring physical[9,17,18], biological[19] and social ecological variables, nitrosamine formation in vivo would represent one important causative factor amongst the clusters of variables (carcinogens, co-carcinogens, risk factors) acting on the stomach of man.

In Antofagasta Province, northern Chile, a chronic exposure to high concentrations of arsenic in drinking water[19] must be considered as an environmental risk for malignant tumours of the skin, liver, and probably of the lung. Two cases with multiple squamous-cell carcinomata of the skin induced by dietary arsenic were reported[19]. For low-income Chileans, the possible co-carcinogenic effect of foods prepared with preheated fats[20,21] must be considered as well.

Summary. Mean age-adjusted death rates for gastric cancer in Chile for 1960, 1962 and 1964 were associated with exposure to sodium nitrate and nitrates ($NaNO_3$ and KNO_3) used as fertilizers, by province. The general population exposure to $NaNO_3$ and nitrates exhibited correlation coefficients of 0.745 and 0.718 with the death rates, respectively.

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Fibrin Stabilizing Factor (F XIII) and Collagen Polymerization

The fibrin stabilizing factor or clotting factor XIII performs a twofold function: 1–It induces the stabilization of fibrin clots by transamidation bonds. 2–It promotes the healing of wounds. In this respect, the only mechanism of factor XIII known up to now was its improving effect on fibroblast culture. We have shown that activated factor XIII (factor $XIII_a$) also causes the formation of stable bonds between various chains of the collagen molecule, thus generating a collagen polymerization process different from the one which occurs in the absence of factor XIII. This modification in the collagen polymerization might possibly account for the defective healing of wounds observed in patients with hereditary factor XIII deficiency.

Materials and methods. Factor XIII: Behringwerke factor XIII* prepared from placenta, used for therapeutic purposes. The factor XIII activity of 1 mg of the substance is identical with that of 3 ml of normal plasma[1] Thrombin Behringwerke: 50 units/ml solution in 0.2 *M* $CaCl_2$, 0.15

* Préparation 1331: factor XIII Concentrate Behring (lab. Hoechst-Paris).
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Table I. Collagen polymerization and factor XIII.

		Tests			Controls			
Factor XIII Activation	Factor XIII	4 mg	4 mg	4 mg	—	—	—	—
	Michaelis buffer (pH 7.4)	4 ml	4 ml	4 ml	4 ml	4 ml	4 ml	4 ml
	Thrombin calcic	0.4 ml	0.4 ml	0.4 ml	0.4 ml	—	—	—
	Saline solution	—	—	—	—	0.4 ml	0.4 ml	0.4 ml
Incubation during 30 min at 37 °C	Addition of	—	GME (100 mg)	MIA (50 mg)	—	GME (100 mg)	MIA (50 mg)	—
Incubation during 15 min at room temperature								
Collagen polymerisation	Collagen solution (ml)	1.1	1.1	1.1	1.1	1.1	1.1	1.1
		Incubation during 30 min at 37 °C and 1 h at room temperature Centrifugation						

Table II. Variation of quantity of collagen polymerized after Factor $XIII_a$ addition. Correction by MIA or GME

Collagen polymerized	Collagen polymerized (%)
in the presence of:	
Factor $XIII_a$	13
Factor $XIII_a$ + GME	45
Factor $XIII_a$ + MIA	45
Thrombin	46
GME	45
MIA	46
without any addition	45

M KCl. Calf skin collagen: Stago (Asnières-France). The reagent contains acid-soluble collagen. The equivalent of 6 mg of collagen is dissolved in 5 ml of distilled water. Glycine methyl ester hydrochloride (GME): Sigma. Sodium iodo acetate (MIA): Fluka.

The level of MIA or GME necessary to induce inactivation of factor $XIII_a$ was determined by clot dissolution test, using monochloracetic acid as described by Josso[2]. The quantities of inhibitor were doubled in the collagen polymerization test.

Collagen polymers were obtained during incubation of acid soluble collagen with buffer solution at pH 7.4. In those conditions, collagen was precipitated as a fibrous mass. The same experiment was carried out in the presence of factor $XIII_a$, and factor $XIII_a$ previously incubated with MIA or GME. Control tests were performed by polymerizing collagen in the presence of thrombin, MIA or GME. The method used is summarized in Table I. The polymerized collagen precipitates were carefully washed 3 times with NaCl 0.15 *M* and then – either dissolved within 18 h in a solution of 9 *M* urea containing 3% SDS in 0.04 *M* sodium phosphate, at pH 7.3 and at 37 °C, then submitted to 3% polyacrylamide gel electrophoresis in the presence of urea and SDS, as described by SCHWARZ[3]. The final protein concentration in each resulting solution was determined by the LOWRY[4] method – or examined by contrast phase microscopy.

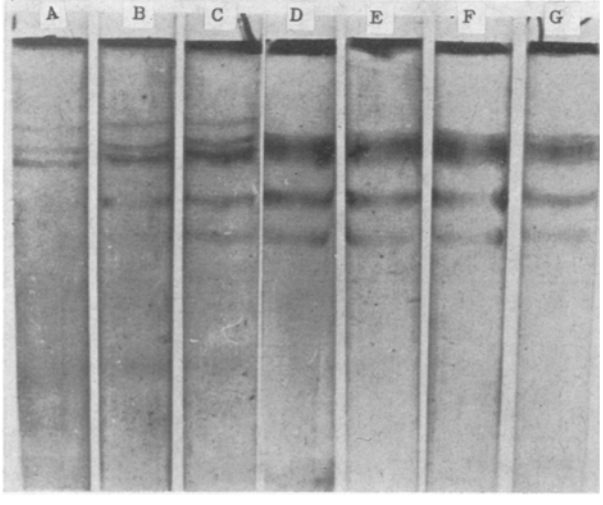

Fig. 1. Polyacrylamide gel-SDS electrophoresis of collagen polymerized in the presence of A) factor $XIII_a$; B) factor $XIII_a$ + GME; C) factor $XIII_a$ + MIA; D) thrombin; E) GME; F) MIA; G) buffer.

Results. 1. Quantity of polymerized collagen. Table II shows that the quantity of collagen precipitated as a fibrous mass, during incubation at 37 °C and neutral pH, differs according to the substance added: Factor $XIII_a$ Factor $XIII_a$ incubated with GME or MIA, Thrombin, MIA or GME.

2. Polyacrylamide gel electrophoresis with SDS (PAG electrophoresis) (Figure 1). Collagen polymerized in the absence of factor XIII, with or without thrombin, is dis-

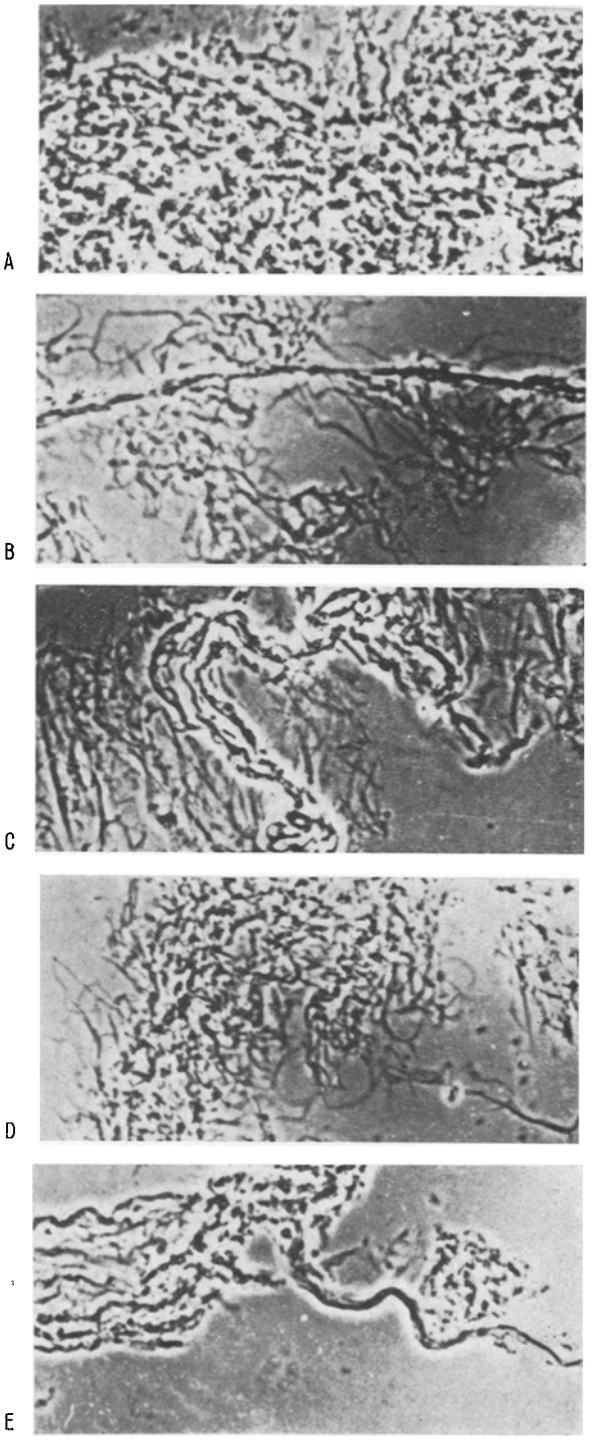

Fig. 2. Phase contrast microscopic examination of collagen polymerized in the presence of A) factor $XIII_a$; B) buffer; C) thrombin; D) factor $XIII_a$ + MIA; E) factor 13_a + GME.

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.

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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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