Summary. A distinct distribution of activated SMC could be demonstrated in atherosclerotic plaques and the neighbouring media of human beings. An increased proliferation was found in the younger age group and generally in the marginal regions of the plaques. The occurrence of activated SMC is thought to be a sequel of metabolic hypoxic damages. A high frequency of activated SMC is a bad prognostic sign in surgical specimens indicating a tendency for proliferation and occlusion.

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Further Evidence of a Positive Correlation Between Exposure to Nitrate Fertilizers ($NaNO_3$ and KNO_3) and Gastric Cancer Death Rates: Nitrites and Nitrosamines

Chile and Japan have the highest age-adjusted mortality rates per 100,000 population for stomach cancer in the world[1]. A survey of 15,300 necropsies performed at Santiago[2] has confirmed the high death rates. The relative frequency of stomach carcinoma amongst males with cancer ranked first with 20.60% and amongst females ranked second with 10.05% (ZALDÍVAR, unpublished observations). The morbid anatomy of gastric cancer in Chileans has been previously described[3-6]. The regional variation of oesophageal and gastric cancer in Chile was reported[7,8]. The predominantly agricultural provinces, which have the highest consumption of nitrate fertilizers, showed the highest death rates for stomach cancer. To explain this fact, a hypothesis of a nitrosamine synthesis in vivo in the stomach was advanced[8]. Recently, an association between exposure to sodium nitrate and gastric cancer mortality for 1960 was reported[9,10].

To study further this environmental problem, more stable death rates for stomach cancer in the 25 Chilean provinces were used (mean age-adjusted death rates per 100,000 pop. for 1960, 1962 and 1964), relating this variable with sodium nitrate exposure as well as with exposure to nitrates ($NaNO_3$ and KNO_3).

Material and methods. The figures on the general population and farm workers by province were taken from the 13th National Population Census. Numerical data on metric tons of nitrates used by province for 1960–61 were provided by the Chemical & Mining Society of Chile. Gastric cancer death rates per 100,000 pop., standardized for age for 1960, 1962 and 1964 by province, were taken from a previous communication[8]. The exposure to nitrate fertilizer in each province was expressed as metric tons of $NaNO_3$ or nitrates per person (general population) or per farm worker (agricultural pop.). The index of general population exposure was obtained by dividing the metric tons of fertilizer for each province by the number of persons in the province. The index of farm worker exposure was obtained in a similar way; the metric tons of fertilizer used in each province were divided by the number of farm workers in the province.

Results and discussion. The regression of death rates on the general population exposure to sodium nitrate showed a highly significant association ($p < 0.00005$). The equation of the regression line is $y = 37.01 + 910.2\,x$, where y is mean age-adjusted death rate for stomach cancer and x is metric tons of sodium nitrate per person. The correlation coefficient was 0.745. The regression of death rate on the farm worker exposure to sodium nitrate exhibited a significant association ($p < 0.0001$). The regression equation is $y = 35.87 + 154.0\,x$, where y is mean death rate and x is metric tons of sodium nitrate per farm worker. The r-value was 0.714. When the death rate was regressed on the general population exposure to nitrates, a significant association was found ($p < 0.0001$). The equation is $y = 36.77 + 454.0\,x$, where y is mean death rate and x is metric tons of nitrates per person. The correlation coefficient was 0.718. The regression of mortality rates on the farm worker exposure to nitrates showed again a significant association ($p < 0.0003$). The equation is $y = 35.53 + 74.88\,x$, where y is mean death rate and x is metric tons of nitrates per farm worker. The r-value was 0.668. The behaviour of the 4 independent variables in the regressions was quite similar, since the r-values were close to each other.

As previously postulated[8-10], the most probable explanation for this relationship is the nitrosamine formation in vivo. According to this hypothesis, nitrates undergo reduction in plants and are transformed into nitrites by nitroreductases present in many parasitic and saprophytic bacteria. Chilean farm workers would be exposed to dietary nitrates and nitrites, primarily from water supplies. The general population of Chile is exposed to nitrates and nitrites from food additives (meat products) and vegetables. SANDER[11] estimated a mean daily intake of nitrite in man of 22 µmoles, equivalent to 1.5 mg of $NaNO_2$. Secondary amines (R′–NH–R″), such as piperidine formed by heating from cadaverine present in partially decayed meat and fish, and pyrrolidine formed by heating from putrescine present in such foodstuffs, may be formed during pyrolysis of protein and therefore during cooking of protein food. Thus, ingested nitrite and certain secondary amines (piperidine, pyrrolidine) may lead to the in vivo formation of carcinogenic nitrosamines. Many amines, including pyrrolidine, have been detected in wine[12,13], a dietary item consumed in considerable

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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						