presence of an α,β-unsaturated δ-lactone grouping at λ_{max}^{EtOH} 220 nm ($\varepsilon = 10{,}000$). Its IR absorption supported this, showing bands at ν_{max} 1765 cm^{-1} (γ-lactone) consistent with related norditerpene lactones[2,5,6]. The PMR-spectrum of **3** closely resembled that of nagilactone G (**2**), podolide (**5**)[7], and podolactone D (**6**)[5]. The multiplet between δ 5.86 and δ 5.92 in (**3**) was assigned to the H-1 and H-2 protons due to a close similarity to shift values reported for H-1 and H-2 of (**6**). Additional support for the olefinic assignment of H-1 and H-2 was obtained from the chemical shift observed for the H-20 protons of (**3**) found at δ 1.24 which closely correlated to that at δ 1.15 for (**6**)[8]. In comparison the signals for the C-20 protons of (**5**) were found further upfield at δ 1.03. The chemical shift value of the C-18 3 proton singlet at δ 1.35 discounted possible positioning of unsaturation between C-2 and C-3 thus establishing its position between C-1 and C-2 as in **6**. TLC on C_{18} reversed-phase silica gel, using methanol: water: acetonitrile (5:3:2) as developing solvent, differentiated the ring A double bond isomers milanjilactone A (R_f 0.44, **3**) and podolide (R_f 0.38, **5**).

2 pairs of 3 proton doublets at δ 1.08 and δ 1.10 indicated the presence of an isopropyl grouping at C-14. Protons H-5, H-6, H-7, H-11 and H-14 were assigned by analogy from their shift values and splitting patterns (table).

Milanjilactone B (**4**), m.p. 220–222 °C, $C_{19}H_{22}O_4$ (M^+ m/e 314.151) showed UV-absorption which supported the presence of a dienolide system identical to that contained in nagilactone F (**1**) at λ_{max}^{EtOH} 257 nm ($\varepsilon = 13{,}000$). Its IR-spectrum was consistent with this showing bands at ν_{max} (KBr) 1685 and 1610 cm^{-1} for the δ-lactone and 1760 cm^{-1} for the γ-lactone. An isopropyl grouping and the 2 quaternary methyl groups were indicated by signals in the PMR at δ 0.98 (3 H, d, 6.8 Hz), 1.20 (3 H, d, 6.8 Hz), δ 1.24 (3 H, s) and δ 1.38 (3 H, s). Further analysis revealed the couplings of H-6 and H-7 and H-14 ($J_{6,7} = J_{6,14} = 1.7$ Hz)[5] analogous to (**1**) in addition to a long range coupling between H-7 and H-11 (J = 1.7 Hz). The chemical shifts for the H-20 and H-18 protons further supported the presence of the C-1, C-2 double bond, with the H-18 proton singlet at δ 1.38 and the H-20 proton signal at δ 1.17 in pyridine-d_5 solution. This latter signal closely correlated with that at δ 1.15 for (**6**)[8]. The stereochemistry of milanjilactone A (**3**) and milanjilactone B (**4**) is proposed based on analogy to nagilactone F (**1**) and podolide (**5**), and is consistent with spectral data.

1 To whom correspondence should be addressed. The authors acknowledge the technical assistance of Ms Teresa K. Lightner. Part 11 in the series 'Potential Antitumor Agents'.

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Involvement of ribonuclease in the interactions of macrophages and fibroblasts in experimental silicosis[1]

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Summary. Decreased ribonuclease activity in the supernatant from silica-treated macrophages is associated with the enhanced protein synthesis in granulation-tissue slices incubated in this supernatant, and with the decreased degradation of polysomes in granuloma slices and of polysomes isolated from the granulation tissue. The phagocytized silica particles adsorb ribonuclease and perhaps other proteins and thus remove them from the macrophage supernatant.

The treatment of peritoneal macrophages with SiO_2 liberates from their subcellular particles a soluble agent which stimulates collagen synthesis in slices from experimental granulation tissue[2,3]. We recently found that the SiO_2-released macrophage factor maintains the stability of polysomes in the incubated slices, and that the silica-treated macrophages enhance the cell-free synthesis of proteins by polysomes from experimental granuloma[4]. Several reports[5] have suggested that ribonuclease (RNase) activity plays a key role in the turnover of polysomes. The purpose of this study was to explore whether there is a correlation between the RNase activities of SiO_2-treated macrophage preparations and the synthesis of collagen and other proteins in incubated slices or in polysomes of experimental granulation tissue.

Materials and methods. Unstimulated macrophages were harvested from rats by washing the peritoneal cavity with 0.9% NaCl solution containing heparin and the 7000/500 × g sediment prepared as described earlier[3]. The sediment from 4–5 × 10^6 macrophages was suspended in 1 ml of the incubation medium and divided into 2 portions to be incubated with and without SiO_2 (0.75 mg/ml Dörentrup Quartz DQ 12) at 37 °C overnight. The 20,000 × g supernatants of the sample were divided into small aliquots and stored at − 70 °C.

Granulation tissue was induced by s.c. implantation of viscose-cellulose sponge (Kongsfoss Fabrikker A/S, Oslo 2, Norway) into 3-month-old female albino rats[6]. Polysomes were prepared from 14-day experimental granulation tissue

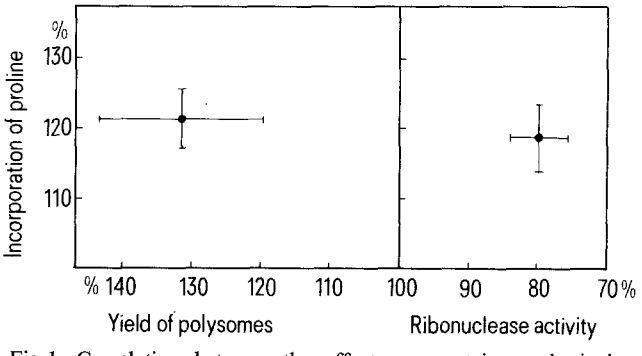

Fig. 1. Correlation between the effects on protein synthesis in granuloma slices and the polysome yield (left) and between the effects on protein synthesis and the RNase activity (right; granuloma polysomes as substrate) of silica-treated macrophage supernatant. The values are expressed in percent of the relevant control values with untreated macrophages (= 100%). The means ± SEM are given (n = 14).

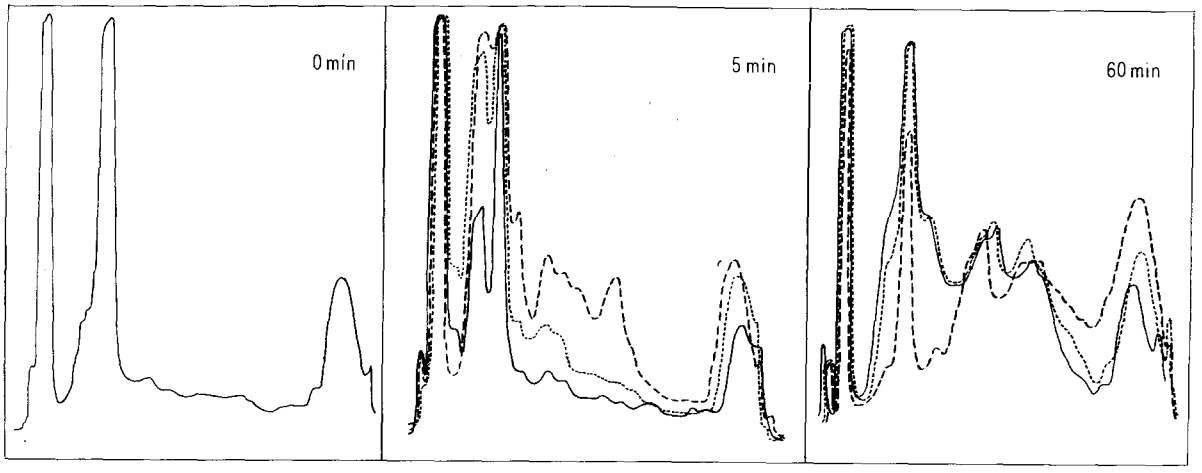

Fig. 2. Autodegradation of polysomal RNA from granulation tissue and the effects of control and silica-treated macrophage supernatants on the degradation pattern. ——, No macrophage preparation added (autodegradation with polysomal RNase); ---, the supernatant of control macrophages added; and ....., the supernatant of silica-treated macrophages added. The densitometric recordings of gel-electrophoretic strips are shown.

according to Palmiter[7]. About 500 mg of granuloma slices was incubated in 3 ml of the 20,000 × g supernatant of SiO_2-treated or control macrophages and 20 µCi [^{3}H]proline for 3 h as described by Aalto et al.[8]. The radioactivities in total protein and in hydroxyproline were estimated according to Juva and Prockop[9].

Ribonuclease activity was determined according to Liu et al.[5]. The reaction mixture contained, in a final volume of 300 µl, 50 µl of 1 M tris-HCl buffer (pH 7.8), 50 µl of 0.02% bovine serum albumin (BSA; Sigma; Cat. No A-4378, Lot 83C-8090) and 50 µl of 2% yeast RNA-solution. The macrophage preparations were usually added in 100 µl of Krebs-Ringer-Hepes buffer.

The disc electrophoresis of RNA was carried out in 3% of acrylamide gels containing sodium dodecyl sulphate SDS (2 g/l in the buffer) at room temperature[10]. After the run, RNA was stained 10 min in methylene blue solution (2.3 ml concentrated acetic acid, 13.5 g sodium acetate and 0.5 g methylene blue in a total volume of 250 ml) and destained overnight in water. The stained gels were scanned with a Perkin-Elmer UV-VIS 139 spectrophotometer at 570 nm with the aid of an adaptor made in our workshop and a Servogor RE 514.9 recorder.

Results. 1st, we found a correlation between the increased protein synthesis and the increased polysome yield in the granulation tissue slices that had been incubated in the 20,000 × g supernatant of silica-treated macrophages (figure 1); 2nd, an inverse correlation between the changes in protein synthesis and the RNase activities of the same supernatants.

The macrophage preparation splits ribosomal RNA as an endonuclease (figure 2). The final pattern of ribosomal RNA-fragments obtained by incubation with the preparation from SiO_2-treated macrophages resembles that where no macrophage preparation had been added at all. The gel-electrophoretic patterns of the breakdown products by the RNase from macrophages differ from those obtained by the autodegradation of fibroblast polysomes, especially in the ranges of 18-28S and 8-12S.

The macrophage ribonuclease has an optimum for Na^+ concentration (10 mM), but Mg^{2+} and Ca^{2+} are inhibitory even at low concentrations. Therefore, the addition of 5 mM EDTA increased the RNase activity of macrophage preparations slightly, although the proportional effects of SiO_2-pretreatment persisted. The RNase activity was sensitive to repeated freezing and thawing, resembling in this respect the silicosis-factor[3].

The present results can be understood by presuming that the release of the RNase activity from the 7000 × g sediment of the macrophages is suppressed by SiO_2. Silica particles adsorb RNase and perhaps other proteins, which are then removed by centrifugation from the supernatant (table).

The SiO_2 preparations adsorbed the RNase activity from the macrophage preparation (figure 3) with a rate dependent on the amount of SiO_2 and the concentration of the macrophage preparation in the mixture. The adsorption of the macrophage RNase to SiO_2 is not linear.

Discussion. According to Kozin and McCarty[11] silica crystals adsorb proteins on their surfaces. Summerton et al.[12] used this phenomenon to explain the membrane destruction by silica, as suggested already by Nash et al.[13].

Adsorption of ribonuclease by silica particles during the incubation of subcellular particles from macrophages

Addition	RNase activity after incubation (mean ± SEM)	
	Total	in 20,000 × g supernatant
None	100.0	84.2 ± 4.5
Silica particles	100.0	56.2 ± 6.7

The 7000/500 × g sediment of homogenized macrophages was incubated with silica as described. 4 independent experiments were made; $p < 0.005$.

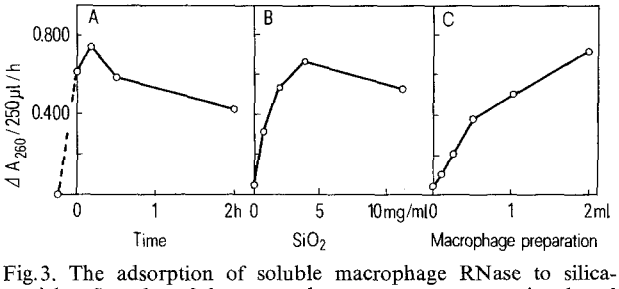

Fig. 3. The adsorption of soluble macrophage RNase to silica-particles. Samples of the macrophage supernatant were incubated with silica at 37 °C as follows: *A* 2-ml samples with 1.5 mg silica for 0-2 h; *B* 1.0-ml samples with 0-11.25 mg silica for 10 min; *C* samples of 0-2.0 ml filled with Krebs-Ringer-Hepes-buffer to a total volume of 2 ml, with 1.5 mg silica for 10 min. After incubation the samples were centrifuged at 20,000 × g for 15 min at +4 °C and the sediment was suspended in 0.5 ml of water. The RNase activity in the sediment was determined.

We find the RNase activity to be decreased in the supernatant of SiO_2-treated macrophages. Presumably, the SiO_2 particles are first bound to the membrane of the macrophage and then phagocytized, at which stage they adsorb and immobilize the ribonuclease protein. The less active the macrophage RNase is, the more stable are the polysomes in the fibroblasts and, hence, the more incorporation there is of labelled amino acids. When RNase in the supernatants from control and SiO_2-treated macrophages is inactivated by freezing and thawing, the incorporation levels increase and the difference levels off. The granulation-tissue fibroblasts also contain polysomal ribonuclease[14].

We suggest that the fibrogenic factor and the inactivation of macrophage RNase, and possibly the preservation of macrophage RNA, are mutually involved, so that the polysomal system in the fibroblasts is stabilized and its capacity thus increased. The physiological correlations of the decreased RNase activity of the macrophages may be sought in the metabolism of nucleic acids in the adjacent fibroblasts.

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Retinol: predominant form of vitamin A in corpus luteum

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Summary. We could not confirm the results of a previous research group[2,3] which stated that retinal (vitamin A aldehyde) is present in corpus luteum of bovine origin. On the contrary we establish the occurrence of retinol (vitamin A alcohol) in both human and bovine corpora lutea. Identity of the compound is confirmed by its chromatographic behavior on high performance liquid chromatographic systems (adsorption and reversed phase) and by spectrometric analysis (UV, fluorimetry).

It is generally accepted that vitamin A plays an important role in reproduction. Both the formation of spermatozoa and the maintenance of pregnancy are influenced by vitamin A[4].

Provitamins A (see also β-carotene) are present in corpus luteum in relatively high concentrations[5,6]. The presence of retinal (vitamin A aldehyde) was reported in corpus luteum of bovine origin[2] but the authors claimed the absence of retinol (vitamin A alcohol). That retinal, but no retinol should be present in a human tissue is a puzzling anomaly as most other tissues, with the exception of the eye, do not contain retinal[7].

We wanted to know if human corpus luteum had the same composition of vitamin A derivatives. Therefore we analyzed corpora lutea dissected from surgically removed ovaries of humans.

Materials and methods. All reagents were of analytical grade and were used as received. All-trans-retinol and all-trans-retinal were obtained from Eastman Kodak Co. All experiments were carried out with a Varian 8500 (Varian, Palo Alto, Ca.) pump and a Varichrom multiple wavelength detector. The injection device was from the sample loop type. Valco UHPa-N60 equipped with a 50 µl loop.

Columns were home packed using a slurry technique[8]. 2 different chromatographic systems were used; an adsorption system (column, 15×0.2 cm inner diameter, 5 µm RSIL silica (RSL, St. Martens-Latem, Belgium); mobile phase, 0.25% (v/v) isopropanol in n-hexane; flow, 1.0 ml/min; detection, absorbance at 330 nm), and a reversed phase system (column, 25×0.46 cm inner diameter, 5 µm Lichrosorb RP 8 (Merck AG, Darmstadt, FRG); mobile phase, 10% (v/v) water in methanol; flow, 2.5 ml/min; detection, absorbance at 330 nm). For quantitative HPLC measurement conditions were used as in our previously reported serum retinol determination[9].

UV-VIS absorption spectra were run on a Pye Unicam SP 1800 (Pye Unicam, Cambridge, England) double beam spectrophotometer. Uncorrected fluorescence spectra were recorded with an Aminco-Bowman SPF (American Instr. Co, Silver Spring, Maryland) spectrophotofluorimeter. Tissues were homogenized with a Virtis 23 (Div. of Cenco, Gardiner, N.Y.) blade homogenizer at 23,000 rpm. Tissue extracts were concentrated under reduced pressure using a Büchler Rotary Evapo-Mix (Büchler Instr., Div. Searle Diagn. Inc., Fort Lee, N.Y.).

Corpora lutea were dissected from surgically removed ovaries of humans and bovine corpus luteum was obtained after slaughtering. The samples were immediately frozen after dissection and kept at -18 °C until analyzed. Prior to analysis the corpus luteum was thawed, washed, patted dry and weighed.

1 g of wet corpus luteum was homogenized for 10 min in 10 ml of an ethanol/water (1:1, by volume) mixture. The homogenate was extracted twice with 10 ml n-hexane. The combined organic layers were evaporated under reduced pressure, and redissolved in the mobile phase of the chromatographic system used. An aliquot was injected on the adsorption system and another on the reversed phase system.

For quantitative measurements a modified procedure of a serum retinol determination was used[9]. To 200 mg wet corpus luteum, 3.0 ml C_2H_5OH and 50 µl of an internal standard solution were added [internal standard = all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8 tetraenol]. The mixture was homogenized for 10 min and centrifuged. An equal volume of H_2O was added to the