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.

1 For financial support we are grateful to the Association of Finnish Life Assurance Companies and to the Medical Research Council in Finland.
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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

supernate and after mixing the extract was divided into 2 equal parts. To 1 part 50 ng of all-trans-retinol dissolved in methanol was added. Both parts were then extracted with 3.0 ml of n-hexane. The hexane layer was concentrated, redissolved in 200 µl n-hexane and 50 µl was injected on the adsorption column.

Results and discussion. Qualitative analyses. An aliquot of the corpus luteum extract analyzed on the adsorption column gave a relatively clean chromatogram with 1 major peak eluting at 9.8 min (k′=23.0) (figure 1,A). No peak could be detected at the elution place of retinal (k′=3.0). Our chromatographic system was made very selective for the separation of retinal and retinol. A selectivity factor (α) of 7.7 is obtained under the conditions used. If retinal had been present in the concentration of 1 µg/g corpus luteum as stated by Austern[2] a sharp peak should have been seen. This is demonstrated in figure 1,B where corpus luteum was spiked with 1 µg/g all-trans-retinal. No peak eluted at the elution volume of retinal in a non-supplemented extract (figure 1,A).

A 2nd aliquot of the non-supplemented corpus luteum extract was injected on the reversed phase column. The chromatogram showed 1 major peak eluting at 3.65 min (k′=2.1). The capacity ratios (k′) of the unknown peak (X) correspond with those obtained for an all-trans-retinol standard; this in both chromatographic systems.

UV and fluorescence analyses were performed on the fraction of column eluates containing the unknown peak. An absorption maximum (λ_{max}) at 325 nm and an excitation (λ_{exc}) and emission (λ_{em}) maximum at 330 nm and 475 nm, respectively, were found. The λ_{max}, λ_{exc} and λ_{em} values agree with values reported in the literature for all-trans-retinol[10,11].

All the above chromatographic and spectrometric results prove that corpus luteum contains retinol. These data are in contradiction with results obtained by another research group[2] in which they stated that retinol was absent but that retinal was present at a concentration of 1.2 µg/g bovine corpus luteum. As we investigated human tissue where the other group employed bovine samples, we subsequently analyzed a corpus luteum of bovine origin. However, we could not demonstrate a species difference.

Quantitative analysis. Peak height ratios (height retinol/height internal standard) of the non-supplemented corpus luteum extract and the extract supplemented with 50 ng of retinol were used for quantitative determination of retinol (figure 2,A and B). 5 human and 1 bovine corpus luteum were analyzed. Values ranged from 0.27 to 1.78 µg/g for humans whereas a value of 0.29 µg/g was found for the bovine sample.

The use of high performance liquid chromatography (HPLC) allowed us to perform the analyses without employing saponification and special colour reactions. Thus the production of artefacts could be avoided. The high selectivity of the systems enabled us to eliminate the puzzling anomaly that retinal was present in a tissue as

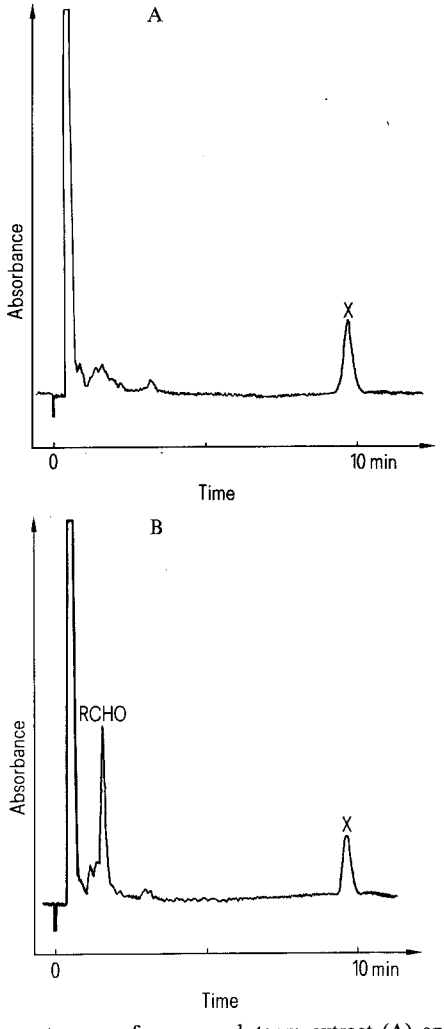

Fig. 1. Chromatogram of a corpus luteum extract (A) and of the supplemented corpus luteum extract (1 µg retinal/g) (B) on the adsorption column. Conditions: column, 15×0.2 cm inner diameter, 5 µm RSIL silica; mobile phase, 0.25% isopropanol in n-hexane; flow, 1.0 ml/min; detection, absorption at 330 nm. Peak identity: endogenous retinol (X) and retinal (RCHO).

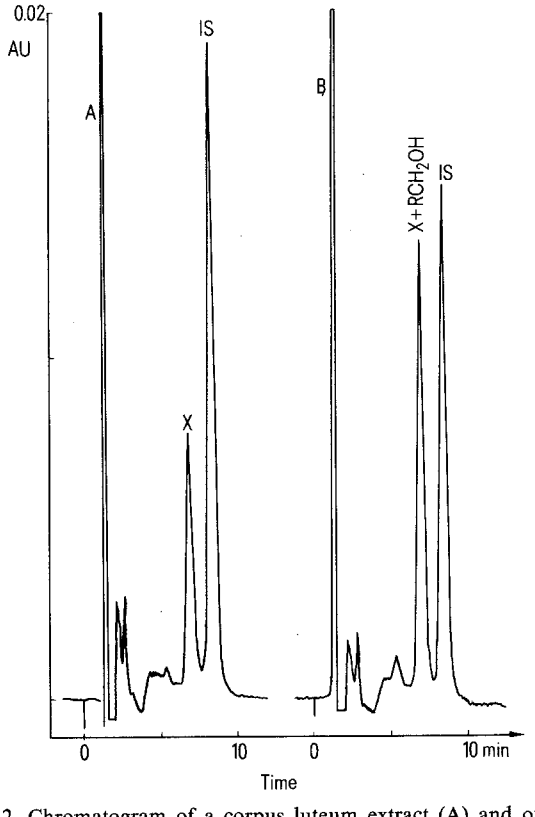

Fig. 2. Chromatogram of a corpus luteum extract (A) and of the supplemented corpus luteum extract (with 50 ng retinol) (B). Conditions: same as figure 1 except mobile phase, n-hexane/CH_2Cl_2/isopropanol (200/50/1.75); flow, 0.25 ml/min. Peak identity: endogenous retinol (X), internal standard (IS) and endogenous retinol + supplemented retinol (X + RCH_2OH).

stated in the literature[2] and to identify retinol in the extracts of corpora lutea.

1 Acknowledgments. The authors are indebted to Prof. D. Vandekerckhove and Prof. F. Derom, University of Gent, Faculty of Medecine, for supplying human corpora lutea.

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Amaninamide, a new toxin of *Amanita virosa* mushrooms[1]

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Summary. Amaninamide, a toxin closely related to the family of amatoxins, was found exclusively in *Amanita virosa* mushrooms. It differs from the well known toxin α-amanitin in that it lacks the 6′-hydroxyl group of the tryptophan unit, and from the toxin amanin found in *Amanita phalloides* by the presence of a carboxamide group instead of a carboxylic acid group.

When methanolic extracts of *Amanita virosa* (FR.) mushrooms were analysed by chromatographic procedures[2], some specimens contained a toxin absent in analogous separation procedures of *A. phalloides* mushrooms. This toxin shows the typical UV-absorption spectrum of α-indolylsulfoxides, as known from the semisynthetic phalloidinsulfoxides[3] as well as from the naturally occurring amanin[4]. However, in chromatographic behaviour it proved dissimilar to either of these compounds. We succeeded in identifying the toxin as amaninamide, the amide of the naturally occurring acidic peptide amanin. Comparison of the toxin with authentic semisynthetic samples of amaninamide provided a direct structural proof.

Materials and methods. Amanita virosa mushrooms were obtained either from Austria (a generous gift of M. Moser, Innsbruck) or from North America (collected by B. Witkop and one of us, Th.W.). Extraction of the mushrooms, column chromatography and further purification on thin layers were as described previously[5]. Pure amaninamide was obtained by a final column chromatography on LH 20/0.004 M NH_4OH. Analysis of amino acids, including the lactone of hydroxylated isoleucine, was performed according to reference[5]. For TLC on silica (Merck, 60F-254) 3 different solvents were used.: I: chloroform-methanol-water (65:25:4, vol/vol); II: sec.butanol-ethylacetate-water (14:12:5, vol/vol); III: sec.butanol-ammonia (2N) (100:44, vol/vol). High voltage paper electrophoresis, according to Wieland and Pfleiderer, was performed using a buffer pH 6.5 containing acetic acid-pyridin-water (2:20:178, vol/vol). Preparation of amaninamide was either from α-amanitin as previously described[6] or from amanin as follows: 9 mg amanin (ca. 10 μmoles) were dissolved in 5 ml of dry dimethylformamide. After addition of 10 μmoles N-methylmorpholine, the mixture was cooled to −15 °C and 10 μmoles isobutylchloroformate were added. After 20 min,

	R^1	R^2
Amaninamide	H	NH_2
Amanin	H	OH
α-Amanitin	OH	NH_2
O-(1-phenyltetrazolyl)-α-Amanitin		NH_2