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Circulating *C*-terminal propeptide of type I procollagen is cleared mainly via the mannose receptor in liver endothelial cells

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The fate of the circulating *C*-terminal propeptide of type I procollagen (PICP) was studied. Trace amounts of ^{125}I-PICP administered intravenously to rats disappeared from the blood with an initial $t_{\frac{1}{2}}$ of 6.1 min. After 45 min the radioactivity was distributed as follows: liver, 36%; blood, 23%; kidneys, 18%; urine, 20%; spleen, 1%; lungs, 2%; heart, 0.4%. To prevent escape of label from the site of uptake, PICP was labelled with ^{125}I-tyramine cellobiose (^{125}I-TC), which is trapped intralysosomally. With this ligand a serum $t_{\frac{1}{2}}$ of 8.7 min was recorded, and 70% and 20% was traced in the liver and kidneys respectively. The uptake per liver endothelial cell (LEC) was 1000 times that per parenchymal cell and twice that per Kupffer cell. At 1 h and 6 h after addition of ^{125}I-PICP to cultured LEC, 15% and 45% respectively, had been endocytosed. Only ligands for the mannose receptor could compete with PICP for endocytosis. To study whether the same specificity was operative *in vivo*, ^{125}I-PICP was injected along with an excess of ovalbumin, which is known to be endocytosed by the mannose receptor of LEC. The serum $t_{\frac{1}{2}}$ was prolonged from 6 to 16 min, signifying that terminal mannose residues are an important signal for clearance of PICP. In conclusion, these studies show that LEC constitute the main site of uptake of circulating PICP. The uptake is mediated by endocytic receptors which recognize terminal mannose residues.

INTRODUCTION

Type I collagen is the most abundant collagen species in many soft tissues and accounts for more than 90% of the organic matrix of mineralized bone. It is synthesized in the form of a larger protein, type I procollagen, which contains relatively long additional sequences at both ends (Prockop *et al.*, 1979*a*,*b*). These sequences, known as the *N*- and *C*-terminal propeptides of type I procollagen, are removed by two specific proteinases in the extracellular space. Proper cleavage of the precursor-specific parts of the molecule is a prerequisite for the appropriate assembly of type I collagen molecules into collagen fibres (Miyahara *et al.*, 1982).

The *C*-terminal propeptide of type I procollagen (PICP), when cleaved off intact from the procollagen molecule, is found in free form in interstitial fluid, e.g. in healing wounds (Haukipuro *et al.*, 1990) and also in blood, where its concentration is thought to reflect type I collagen synthesis in the body, especially by the skeleton (Parfitt *et al.*, 1987). However, for the correct interpretation of the concentration of PICP in blood, it would also be essential to know the routes along which this compound leaves the circulation.

The metabolic fate of several other connective tissue-derived substances in blood has recently been determined. These include hyaluronan, chondroitin sulphate, collagens, the laminin/nidogen complex and the *N*-terminal propeptide of type III procollagen (PIIINP), all of which are taken up, via at least three different receptor systems, by the endothelial cells of the liver (for a review, see Smedsrød *et al.*, 1990).

The aim of the present study was to establish how PICP is cleared from the circulation. To this end, we studied the kinetics of clearance and organ distribution of radioiodinated PICP injected intravenously into rats. Our results suggest that uptake of PICP is another function of liver endothelial cells and is mediated via receptors that recognize terminal mannose residues.

MATERIALS AND METHODS

Chemicals and animals

Cellobiose, tyramine, cyanoborohydride, collagenase, cyanuric chloride, bovine serum albumin, ovalbumin, mannose, galactose, mannan and 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. [^{14}C]Tyramine hydrochloride and Na^{125}I were from Amersham International, Amersham, Bucks., U.K. and from Institutt for Energiteknikk, Kjeller, Norway. Fibronectin and human serum albumin were kindly donated by Kabi Vitrum AB, Stockholm, Sweden. Gelatinized type I collagen, prepared as described (Miller & Rhodes, 1982), was kindly given by Dr. K. Rubin, University of Uppsala, Uppsala, Sweden. Hyaluronan (Healon) was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Culture medium RPMI 1640, supplemented with L-glutamine (2 mM), gentamicin (200 μg/ml) and fungizone (50 μg/ml), was purchased from Flow Laboratories, Irvine, Scotland, U.K. Hypnorm and Dormicum were from Janssen Pharmaceutica, Beerse, Belgium, and Hoffmann-LaRoche, Basel, Switzerland, respectively. Male Sprague–Dawley rats, fed on a standard diet and weighing about 200 g at the time of the study, were from Charles River, Fulzfeld, Germany.

Purification of PICP

Type I procollagen was first purified from the culture medium of human skin fibroblasts, essentially as described previously (Risteli & Risteli, 1987). The procollagen was digested for 16 h at 30 °C with highly purified bacterial collagenase (Worthington, grade CLSPA), using 0.3 mg of enzyme/litre of original medium volume. The *C*-terminal propeptide was purified from the digest by lectin affinity chromatography on concanavalin A–Sepharose (Pharmacia) in 50 mM-Tris/HCl buffer, pH 7.4, containing 0.5 M-NaCl, 10 mM-*N*-ethylmaleimide, 0.3 mM-

Abbreviations used: KC, Kupffer cells; LEC, liver endothelial cells; PC, parenchymal cells; PICP, *C*-terminal propeptide of type I procollagen; PIIINP, *N*-terminal propeptide of type III procollagen; TC, tyramine cellobiose.

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phenylmethanesulphonyl fluoride and 0.5 mM-$CaCl_2,2H_2O$. The propeptide, which was bound to the column, was eluted with the above solution containing 0.5 M-α-methylmannoside. Further purification was achieved by gel filtration on Sephacryl S-300 (Pharmacia) in 0.2 M-NH_4HCO_3 and by anion-exchange chromatography on h.p.l.c. [column: Protein-Pak DEAE 5PW (Waters, Milford, MA, U.S.A.); mobile phase: 50 mM-Tris-acetate, pH 8.0; flow rate 1 ml/min]. The bound propeptide was eluted with linear gradient of NaCl (0–0.5 M in 60 min). The purity of the protein was verified by PAGE and its identity was confirmed by *N*-terminal sequence analysis (Melkko *et al.*, 1990).

^{125}I-labelling of the tyramine cellobiose adduct and coupling to PICP

The method described by Pittman *et al.* (1983) was used, with minor modifications. A microreaction vessel was coated with 10 μg of 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril. After washing with water to remove non-adherent reactant, 20 nmol of tyramine cellobiose (TC) [synthesized exactly as described by Smedsrød (1988)] in 1 μl of 0.02M-KH_2PO_4 (pH 7.2) and 2 mCi of $Na^{125}I$ (20 μl) were added. The incubation was stopped after 1 h by transferring the mixture to a vessel containing 10 μl of 0.1 M-sodium bisulphite and 5 μl of 0.1 M-KI.

To activate the adduct, 20 nmol of cyanuric chloride (equimolar to the adduct) in 20 μl of acetone and 40 nmol of NaOH in 5 μl of water were added. After 30 s, 60 nmol of acetic acid in 4 μl of water was added to quench further reaction. The resulting activated ^{125}I-labelled TC adduct (10 nmol) was incubated for 1 h at room temperature with 100 μg of PICP in 0.5 M-Tris/HCl, pH 7.2. The mixture was desalted on a PD-10 column (Sephadex G 25; Pharmacia) to remove reactants not conjugated to PICP. The resulting radioactivity incorporated was 10^5 c.p.m./μg of protein. Radioactivity was determined in a Packard 5260 Auto-Gamma scintillation spectrometer.

Direct labelling with ^{125}I

Incorporation of ^{125}I into aromatic amino acids of PICP was carried out by the chloramine-T method using Iodobeads (Pierce Chemical Co., Rockford, IL, U.S.A.). The resulting radioactivity incorporated was approx. 10^6 c.p.m./μg of protein.

Turnover and distribution *in vivo* of ^{125}I-PICP and ^{125}I-TC–PICP

Rats were anaesthetized by intraperitoneal injection of a mixture of Hypnorm and Dormicum, and ^{125}I-PICP (10^7 c.p.m.) or ^{125}I-TC–PICP (10^6 c.p.m.) was injected through the tail vein. Blood samples of 25 μl were collected from the tail tip in calibrated capillary tubes. Samples from rats injected with ^{125}I-PICP were mixed with 0.5 ml of 20% trichloroacetic acid to precipitate non-degraded protein, and radioactivity in the supernatant after centrifugation (acid-soluble radioactivity) was taken as degraded PICP. Radioactivity in blood 1 min after injection was taken as 100%. The abdomen of rats injected with ^{125}I-TC-PICP was cut open after 40 min, and a cannula was inserted into the portal vein. Collagenase perfusion and purification of liver cells were carried out as described below. Radioactivities were measured in solubilizates of cultured Kupffer cells (KC), liver endothelial cells (LEC) and parenchymal cells (PC) (prepared as described below, in 6 cm-diam. cell-culture dishes from Costar, Cambridge, MA, U.S.A.). Cell numbers were assessed by visual counting of glutaraldehyde-fixed cultures in a phase contrast microscope. The organs specified were removed and analysed for radioactivity.

Isolation and cultivation of liver cells

The method for preparation of pure cultures of functionally intact KC, LEC and PC from a single rat liver has been detailed elsewhere (Smedsrød & Pertoft, 1985; Laakso & Smedsrød, 1987). Briefly, the liver was perfused with collagenase, and the resulting single cell suspension was subjected to velocity and density centrifugations to produce pure suspensions of PC and non-PC. Monolayer cultures of pure PC were established and maintained in fibronectin-coated dishes. The suspension of non-PC was a mixture of KC, LEC and stellate cells, and was essentially devoid of PC, red blood cells and debris. Seeding of this cell suspension into dishes coated with glutaraldehyde-treated human albumin, followed by a 15 min incubation at 37 °C, resulted in attachment and spreading of KC only. Unattached cells were transferred to fibronectin-coated dishes to enable attachment and spreading of LEC. The average numbers of cells grown per cm^2 were 5×10^4 KC, 2.5×10^5 LEC and 1×10^5 PC. Contamination of KC cultures by LEC and stellate cells was less than 1% and 10% respectively. LEC cultures were contaminated by less than 1% and 10% KC and stellate cells respectively. Cultures of either cell type, established in 3.5 cm-diam. cell culture dishes (Costar), were maintained in RPMI medium without serum. The identification of the different cell types has been described (Smedsrød *et al.*, 1985*c*).

Binding and endocytosis of labelled ligands by cultured liver cells

Cultures of LEC were washed and supplied with fresh medium containing 1% human serum albumin and labelled proteins. Substances added and duration of experiments are specified in

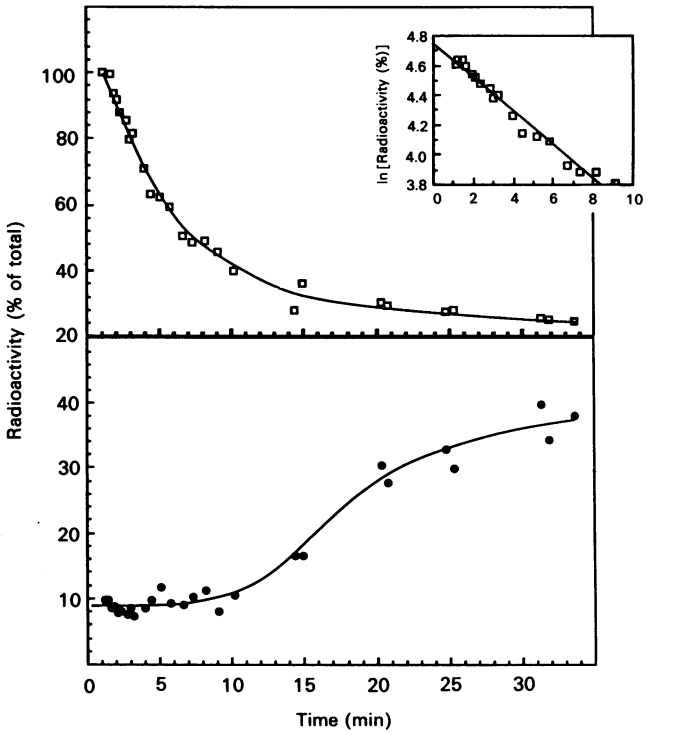

Fig. 1. Uptake and degradation of intravenously injected ^{125}I-PICP

^{125}I-PICP (10^7 c.p.m.; 10 μg) was injected through the tail vein of a rat. Blood samples were collected into tubes containing trichloroacetic acid to precipitate non-degraded proteins. Acid-precipitable radioactivity (intact ^{125}I-PICP, □) measured after 1 min was taken as 100%. Acid-soluble radioactivity (degraded ^{125}I-PICP, ●) is given as a percentage of total radioactivity in the blood samples. The inset is a semilogarithmic plot of survival of PICP in the blood during the first 10 min after injection. The value of serum $t_{\frac{1}{2}}$ was calculated from the slope of the line. In this experiment the $t_{\frac{1}{2}}$ was 6.2 min.

the Results section. Incubations were carried out for various lengths of time at 37 °C to measure endocytosis. Experiments were terminated by transferring the media, along with one wash, to tubes containing 20% trichloroacetic acid, which precipitates only intact undegraded protein. The extent of degradation was determined by measuring the radioactivities in the pellet and supernatant obtained after centrifugation. Cell-associated ligand was determined by measuring the amount of label released by treating washed cultures with a solution containing 1% (w/v) SDS and 0.3 M-NaOH.

RESULTS

Trace amounts of ^{125}I-PICP were injected intravenously into rats to investigate the circulatory survival and the anatomical distribution of this propeptide. Fig. 1 shows that the serum $t_{\frac{1}{2}}$ for the initial 10 min in this particular experiment was 6.2 min, which could be calculated from the semi-logarithmic plot (inset of Fig. 1). Two experiments, including the one presented as Fig. 1, gave a mean $t_{\frac{1}{2}}$ of 6.1 min. Acid-soluble ^{125}I-labelled material started to appear in the blood after 10 min (Fig. 1). The anatomical distribution of the label, obtained by measuring the radioactivity in various organs 45 min after injection (Fig. 2), gives the impression of highest uptake activity in liver and kidneys (36% and 18% of recovered label respectively), whereas the content in blood and urine amounted to 23% and 20% respectively. Uptake in spleen, heart and lungs was as low as about 3.5% combined. Due to effective degradation of eliminated propeptide, and therefore escape of label from the site of uptake, it is unlikely that the anatomical distribution recorded after 45 min correctly reflects the relative importance of these organs in the sequestration of PICP. To overcome the problem of release of label after uptake and degradation, PICP was labelled with ^{125}I-TC, which is trapped intralysosomally after hydrolysis of the carrier PICP. Following intravenous administration of PICP labelled in this manner, a mean $t_{\frac{1}{2}}$ of 8.7 min (S.D. = 0.9; n = 6) was recorded. The results of a typical experiment are shown in Fig. 3. Small ^{125}I-labelled degradation products could not be detected in the blood at any time point. Analysis of the anatomical distribution revealed that with this ligand the liver contained nearly 70% of the recovered radioactivity, whereas about 20% was taken up in the kidneys (Fig. 4). Only insignificant amounts were found in spleen, lungs and heart. The distributions were the same after 2 h and after 24 h (results not shown), suggesting that the ligand was degraded intralysosomally in the organs examined.

Analysis of preparations of purified liver cells after injection of

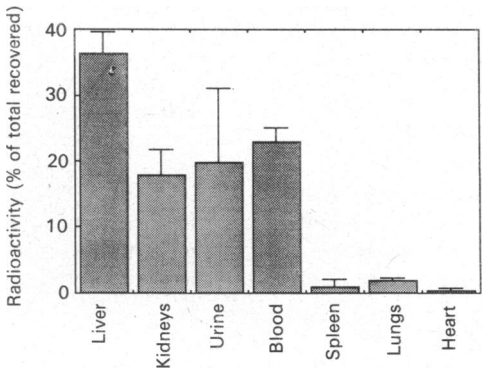

Fig. 2. Anatomical distribution of intravenously injected ^{125}I-PICP

At 45 min after intravenous injection of ^{125}I-PICP (10^7 c.p.m.; 10 μg), organs were analysed for radioactivity content. About 50% of injected dose was recovered in the tissues listed. Results are expressed as percentages of total radioactivity recovered. Bars represent variation between two experiments.

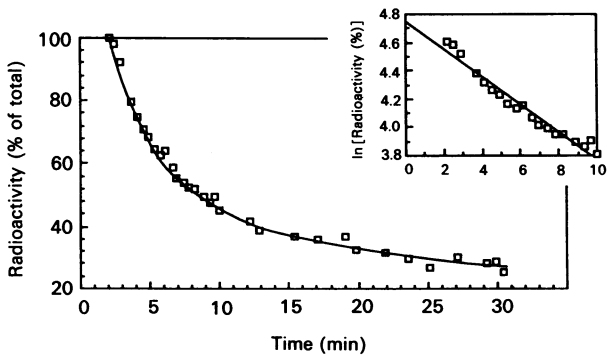

Fig. 3. Removal of ^{125}I-TC–PICP from the circulation

^{125}I-TC–PICP (10^6 c.p.m.; 10 μg) was injected through the tail vein of a rat, and radioactivity in blood samples taken at various time points was plotted against time after injection (see the legend to Fig. 1). ^{125}I-labelled degradation products could not be detected. The serum $t_{\frac{1}{2}}$ as calculated from the slope of the inset was 7.1 min.

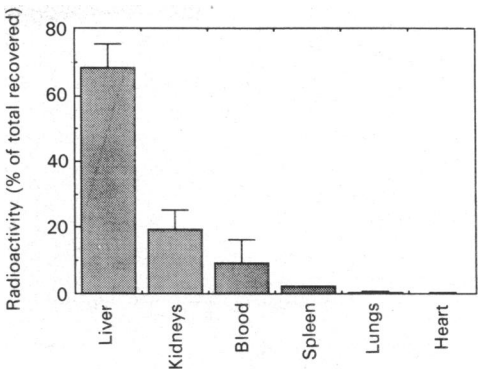

Fig. 4. Anatomical distribution of intravenously injected ^{125}I-TC–PICP

At 30 min after intravenous injection of ^{125}I-TC–PICP (10^6 c.p.m.; 10 μg) the organs were analysed for radioactivity. About 80% of the injected dose was recovered in the tissues listed. Results are expressed as percentages of total radioactivity recovered. Bars represent S.D. (n = 4).

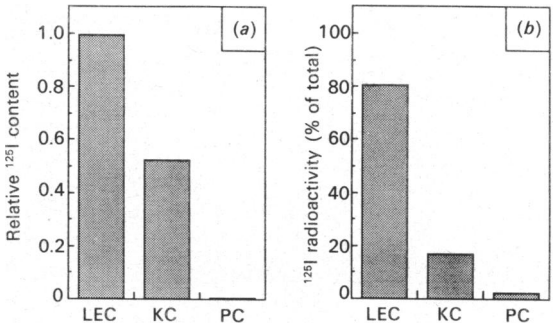

Fig. 5. Hepatocellular distribution of intravenously injected ^{125}I-TC–PICP

At 30 min after intravenous administration of ^{125}I-TC–PICP (10^6 c.p.m.; 10 μg) the cells of the liver were dispersed by collagenase perfusion, and LEC, KC and PC were established in pure cultures. The content of ^{125}I in 10^6 cells is either c.p.m. expressed in relative units, with c.p.m./10^6 LEC equal to 1 (*a*), or is given as % c.p.m. taken up/total cell population in liver (*b*), making use of the fact that the total numbers of KC, LEC and PC in rat liver are present in the proportions 1:2.5:7.7 respectively (Pertoft & Smedsrød, 1987).

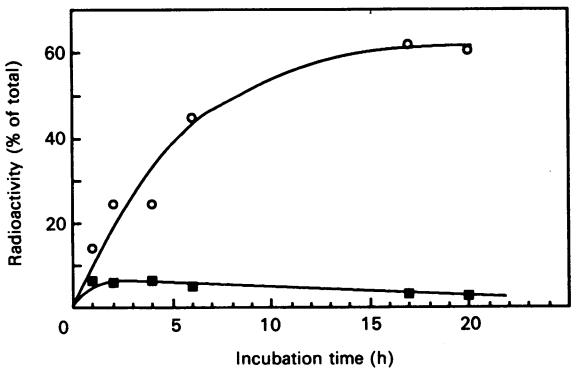

Fig. 6. Kinetics of endocytosis of ^{125}I-PICP in cultured liver cells

^{125}I-PICP (10^4 c.p.m.; 10 ng) was incubated with cultures of LEC in 3.5 cm diam. dishes. Cell-associated (■) and acid-soluble (○) radioactivity was determined after various periods of incubation at 37 °C. Results are means of triplicates.

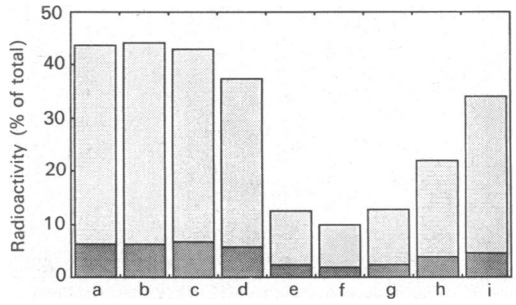

Fig. 7. Specificity of endocytosis of ^{125}I-PICP in LEC

Cultures of LEC were incubated with ^{125}I-PICP (10^4 c.p.m.; 10 ng) for 4 h at 37 °C in 3.5 cm diameter dishes containing 2.5×10^6 cells and 600 μl of medium without inhibitors (*a*), or in the presence of unlabelled gelatinized type I collagen (10 μg/ml) (b); hyaluronan (10 μg/ml) (*c*); 50 mM-galactose (*d*); 50 mM-mannose (*e*); mannan (1 mg/ml) (*f*); mannan (100 μg/ml) (*g*); mannan (10 μg/ml) (*h*); or mannan 1 μg/ml (*i*). ▩, Cell-associated radioactivity; ⊡, acid-soluble radioactivity. Results, given as percentages of total radioactivity, are means of triplicate measurements.

^{125}I-TC–PICP revealed that most of the label was taken up by LEC. One-third was recovered in KC, whereas only negligible amounts were found in PC. The uptake per LEC was 1000 times that per PC, and twice that per KC (Fig. 5). Since the relative cell number distribution in rat liver is 69% PC, 22% LEC and 9% KC (Smedsrød & Pertoft, 1987), it could be estimated that the uptake per cell population in the intact liver was 81% in LEC, 17% in KC and 2.5% in PC.

For more controlled studies of the kinetics and specificity of uptake, pure monolayer cultures of LEC were established. Trace amounts of ^{125}I-PICP added to the cultures were taken up at a constant rate during the first 4 h; thereafter the rate slowed down (Fig. 6). After 20 h, more than 60% of the added ligand had been endocytosed. Degradation was very effective, as shown by the observation that most of the endocytosed ligand was degraded already after 1 h of incubation.

To study the specificity of endocytosis, ^{125}I-PICP was added to the cultures along with an excess of various unlabelled substances known to bind to different endocytosis receptors on LEC. The results show that only mannose (50 mM) or mannan (⩾ 10 μg/ml) could significantly inhibit endocytosis and degradation of the propeptide (Fig. 7). Galactose (50 mM) was only slightly inhibitory, whereas neither gelatinized type I collagen (10 μg/ml) nor hyaluronan (10 μg/ml), both of which are avidly endocytosed via specific receptors on LEC, could inhibit the uptake. These findings suggest that PICP is endocytosed by LEC mainly via mannose receptors. To establish whether this also holds for the situation *in vivo*, ^{125}I-PICP was injected intravenously into rats along with an excess amount of ovalbumin (initial blood concentration about 3 mg/ml), which is known to be taken up mainly by the mannose receptor of LEC *in vivo* (Smedsrød & Einarsson, 1990). The results from two such experiments showed that under these conditions the serum $t_{\frac{1}{2}}$ of the propeptide increased to about 16 min (Fig. 8), suggesting that the mannose receptor on LEC is indeed responsible for the clearance of circulating PICP *in vivo*.

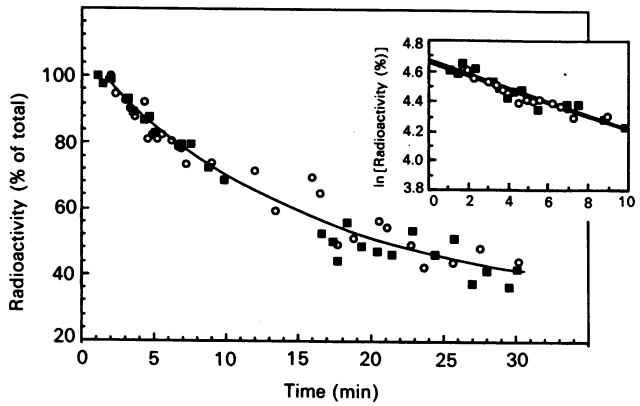

Fig. 8. Inhibitory effect of ovalbumin on elimination of ^{125}I-PICP from the circulation.

^{125}I-PICP (10^6 c.p.m.; 10 μg) and unlabelled ovalbumin (50 mg) were injected through the tail vein of a rat, and radioactivity in blood samples taken at various time points was plotted against time after injection. Results from two experiments are plotted. See the legend to Fig. 1 for additional explanation. The serum $t_{\frac{1}{2}}$ (calculated from the slope of the inset) was 16 min.

DISCUSSION

Based on the notion that the serum level of connective tissue components directly mirrors the release of these substances from the connective tissue, it was first believed that measurement of such molecules in serum would serve as a sensitive non-invasive means to diagnose disorders in which abnormal production or destruction of connective tissue is involved. This view has been modified by the finding that the LEC efficiently eliminate not only hyaluronan (Smedsrød *et al.*, 1984) and PIIINP (Smedsrød, 1988), but also chondroitin sulphate (Smedsrød *et al.*, 1985*a*), gelatinized collagens (Smedsrød *et al.*, 1985*b*; Smedsrød, 1989) and laminin/nidogen (Smedsrød *et al.*, 1989). High-affinity binding mechanisms on LEC recognize these molecules in three distinct groups: (1) hyaluronan and chondroitin sulphate/chondroitin sulphate proteoglycans; (2) gelatinized collagens; (3) PIIINP (for a review, see Smedsrød *et al.*, 1990). Receptors for endocytosis of IgG-immune complexes (Fc-receptor) and mannose-terminated glycoconjugates, both of which are present on LEC, are not involved in the uptake of these connective tissue macromolecules. The specificity of endocytosis of laminin/nidogen is as yet unknown.

The serum $t_{\frac{1}{2}}$ of intravenously injected PICP labelled with ^{125}I in the phenyl rings of the aromatic amino acids was found to be 6.1 min, which is significantly longer than the serum $t_{\frac{1}{2}}$ values observed for the other connective tissue macromolecules taken

up by these cells (Dahl *et al.*, 1988; Smedsrød *et al.*, 1989). About 10 min after administration of ^{125}I-PICP, acid-soluble ^{125}I-labelled material started to appear in the blood, indicating degradation of the protein after its endocytosis and transfer to the lysosomal compartment. The anatomical distribution of label monitored 45 min after injection of ^{125}I-PICP was compatible with the highest uptake by the liver, but the radioactivity recovered in the kidneys amounted to more than 50% of that in liver. Since degradation of endocytosed ligand leads to a constant release of label from the cellular site of uptake, such an experiment underestimates the extent of uptake in the organ(s) which actively endocytose and degrade the ligand. To overcome this problem the distribution experiments were repeated using PICP conjugated with ^{125}I-labelled TC, which is arrested intralysosomally after endocytosis and degradation of the PICP which carried it into the cell. The $t_{\frac{1}{2}}$ obtained with this conjugate (8.7 min) was somewhat longer than obtained with directly labelled ^{125}I-PICP. The reason for this difference is not known. Analysis of radioactivities in various tissues following injection revealed that the liver was the main site of uptake, containing more than 3 times the amount recovered in the kidneys. Significant amounts of label could be found only in these two organs. Therefore, although most of the injected propeptide is sequestered by the liver, uptake in kidneys is also significant. Analysis of radioactivity in pure preparations of the three major types of liver cells after intravenous administration of ^{125}I-TC–PICP disclosed that the population of LEC, containing 80% of the PICP radioactivity in the liver, is the main site of uptake. Uptake in KC represented less than 20% of total liver radioactivity, whereas the PC contained only insignificant amounts.

In order to carry out controlled studies on the kinetics and the specificity of endocytosis of the propeptide, pure monolayer cultures of LEC were given ^{125}I-PICP, and cells and media were analysed for the presence of intact or degraded ligand. If no inhibitors were present, the cells took up and degraded the ligand at an initial speed (during the first 3–4 h of incubation) corresponding to a clearance efficiency of about 0.4 pl of medium/min per cell. Thus a considerably lower rate of uptake was observed for PICP than for any other of the major connective tissue macromolecules known to be taken up by receptor-mediated endocytosis by LEC *in vitro* (Smedsrød *et al.*, 1986). This explains the comparatively long serum $t_{\frac{1}{2}}$ observed *in vivo* for this propeptide. The clearance efficiency decreased with a prolonged time of incubation, and after 20 h the uptake had levelled off when about 60% of added ligand had been endocytosed, probably due to lowering of ligand concentration and exhaustion of the cells. The same phenomenon has been reported for the endocytosis of hyaluronan in cultured LEC (Smedsrød *et al.*, 1984). Degradation proceeds very effectively, as indicated by the observation that the cell-associated radioactivity peaked at about 6% after 1–2 h of incubation, and decreased thereafter.

Inhibition experiments with cultured LEC revealed that mannose (50 mM) and mannan (1 mg/ml) inhibited endocytosis of ^{125}I-PICP by about 75%. No inhibition was observed with gelatinized type I collagen, hyaluronan or galactose when used in concentrations sufficiently high to effectively compete for binding to their respective receptors on LEC and PC. These results support the idea that the mannose receptor on LEC represents the main mechanism of uptake of circulating PICP. To test this hypothesis, ^{125}I-PICP was administered intravenously along with high amounts of ovalbumin, which is known to be avidly endocytosed by LEC via the mannose receptor (Smedsrød & Einarsson, 1990). Monitoring the disappearance of label from the circulation revealed that the $t_{\frac{1}{2}}$ was increased by a factor of almost three. This observation, along with the results from the specificity experiments *in vitro*, strongly suggests that the propeptide is removed from the blood by means of endocytosis mainly via mannose receptors on LEC.

Recent research has assigned major scavenger functions to the population of LEC (Smedsrød *et al.*, 1990). The list of major connective tissue-derived macromolecules cleared by these cells is now extended by PICP, which is endocytosed via the mannose receptor. Uptake of a connective tissue macromolecule via this particular receptor has not been reported until now. Since the mannose receptor recognizes mannose only if it is positioned in the terminal end of the carbohydrate side chain, our result fits well with the findings of Clark & Kefalides (1976) and Clark (1979) that the propeptide contains covalently linked high-mannose-type oligosaccharide, one branch per polypeptide chain.

The serum $t_{\frac{1}{2}}$ of PICP was found to be 6.1–8.7 min. Although this reflects an effective mechanism of clearance, it is considerably slower than the serum $t_{\frac{1}{2}}$ reported for hyaluronan ($t_{\frac{1}{2}} = 0.9$ min; Dahl *et al.*, 1988), gelatinized collagens ($t_{\frac{1}{2}} = 2.2$ min; Smedsrød, 1989), and PIIINP ($t_{\frac{1}{2}} = 2$ min; Smedsrød, 1988). This implies that increased serum levels of hyaluronan and PIIINP, which have been taken as signs of ongoing hepatic fibrosis or connective tissue diseases elsewhere in the body, can actually more easily indicate an insufficient uptake function in LEC, whereas increased serum levels of PICP may serve as a better marker for an increased production of connective tissue.

This study was carried out with rats only. Therefore it may be argued that our findings do not necessarily apply to humans. Nevertheless, data from work with LEC from other species, including humans, signify the same morphology and functional properties of the cells. Moreover, since the recognized substance, namely terminal mannose, is not a species-specific structure, it is highly probable that what we have found in rat concerning the uptake of circulating PICP applies to humans as well.

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