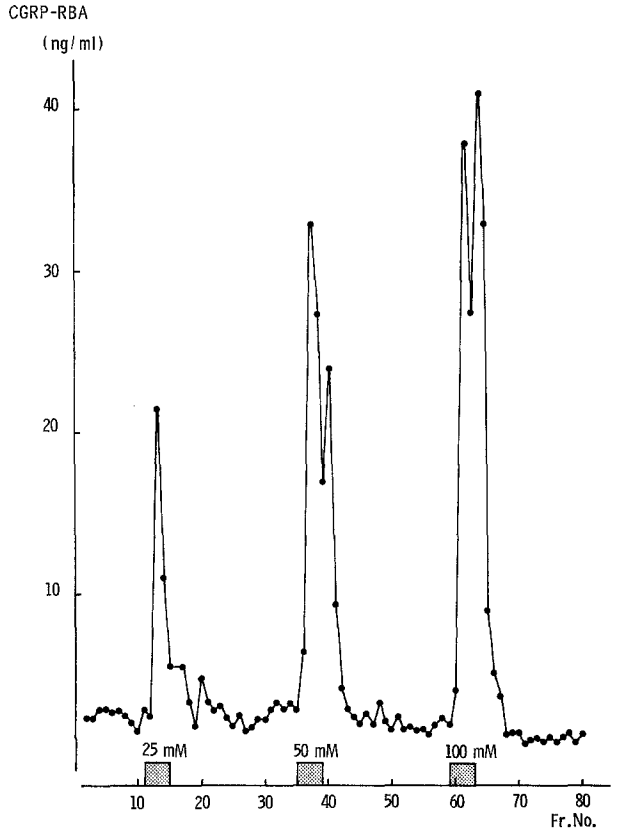

Figure 2. Ca^{++}-evoked hCGRP secretion of perifused human MCT tissue.

secreted concomitantly with the release of calcitonin and somatostatin from the human MCT. Brain et al.[9] recently reported that hCGRP possesses potent vasodilatatory activity in rats and humans[9]. The occasional flushing experienced in patients with MCT may be explained by the transient release of hCGRP[9].
It is tentatively concluded that the abnormally high concentration of hCGRP in the plasma of patients with MCT originates from MCT tissues.

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Inhibition of human pancreatic elastase II activity on human aortic elastin by human α2-macroglobulin

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Summary. Human α2-macroglobulin-human pancreatic elastase II binding were investigated using a homologous substrate, human aortic elastin, in order to test the enzymatic activity. We demonstrated that two moles of α2-M are required to inhibit one mole of HPE_{II} when the enzyme is added to a mixture of elastin and α2-M. In addition, when the elastase-α2-M complex is prepared under some circumstances, it exhibits an elastinolytic activity.
Key words. Pancreatic elastase II; α2-macroglobulin; aortic elastin; human; elastinolysis.

Human pancreatic elastase II ($H.P.E_{II}$) is a protein of 25,000 mol.wt able to hydrolyze insoluble elastin. Plasma α1-antiprotease (α1-AP) and α2-macroglobulin (α2-M) have been shown to inhibit this enzyme[1-3]. The latter protein forms enzymatic complexes with proteinases and in some cases the enzyme may be released from the complex[4-9]. However, recently we have shown that, when homologous reactants were used, the complex between HPE_{II} and α1-AP was able to degrade homologous elastin[10,11]. The interaction between HPE_{II} and α2-M has been studied especially by Gustavson et al.[9] using Benzyloxycarbonyl-alanine-p nitrophenyl ester as a substrate.
We decided to investigate the human α2-M-human $P.E._{II}$ binding using a homologous substrate to test the enzymatic activity. We demonstrate here that two moles of α2-M are required to inhibit one mole of HPE_{II}, when the enzyme was added to a mixture of elastin and α2-M. In addition, when the elastase – α2-M complex is prepared, under some circumstances, it exhibits an elastinolytic activity.

Materials and methods. Human pancreas and aorta were extracted from brain-death patients, together with the kidneys later used for transplantation by the 'monobloc' technique at the University Hospital Center of Bordeaux[12]. Human pancreatic elastase: HPE_{II} was chosen for this study because under physiological conditions, it is the enzyme secreted in greatest quantities by the exocrine region of the pancreas. Several authors have shown that it reaches the general circulation by the lymphatic pathway[13] or by crossing the intestinal barrier[14-16]. The enzyme was purified by the procedure of Largman et al.[17] and labeled with ^{125}Iodine as described in Rabaud et al.[11]. Human aortic elastin was prepared according to the method of Leppert et al.[18]. The fibers were swollen by equilibration in the appropriate buffer.
Human α2-Macroglobulin was purified as previously described[8]. It was kept at 4 °C in a solution buffered by 10 mM Tris HCl 500 mM NaCl, pH 7.2. The protein was homogeneous in polyacrylamide gel electrophoresis and had no detectable ami-

dolytic activity. Moreover, the purity was corroborated by radial immuno-diffusion using M-Partigen immunodiffusion plates (Behringwerke AG, Marburg W., Germany). This method gave an α2-M purity of 99%.

Elastinolysis was determined by measuring solubilized peptides using the technique of Lowry et al.[19]. Although this technique is not very sensitive, it has been preferred to the isotopic method using ^{125}Iodine[20], because elastin does not contain many tyrosyl residues able to react with iodine; moreover, the fibrillar structure restricts the labeling to the periphery of the fibers. Thus, elastinolytic activity was determined after incubation in 5 ml total volume at 35 °C of an elastin suspension (2 mg/ml) in 10 mM Tris-HCl, 1 mM $MgCl_2$ pH 8.6 and various amounts of α2-M (0–150 nM). The reaction was started by adding the elastase solution and was stopped by centrifugation (10,000 g); 0.8 ml aliquots were removed every 3 min and the protein concentration was determined in duplicate from 0.2 ml of supernatant.

Preparation of the α2-M-HPE complex. A solution of α2-M (40 μM) was pre-incubated for 1 h at 37 °C in 10 mM Tris, HCl pH 7.5 buffer containing 150 mM NaCl and 2 mM $CaCl_2$. Then ^{125}Iodine HPE was added dropwise in increasing amounts so that the I/E molar ratios varied from 10 to 0.1. The reaction mixture was dialyzed against 0.1 M acetate buffer pH 6 as described previously for the α1-AP-HPE complex[11]. The mixture was then applied to Concanavalin A-Sepharose, equilibrated with the acetate buffer, to remove the excess of elastase, which is not a glycoprotein and is therefore not retained on Con A-Sepharose. The complexes, which conserved the glycoprotein character and the radioactivity due to elastase, were eluted as previously described for α1-AP HPE[11]. The use of acetate buffer pH 6 was not injurious to α2-M since previous studies have shown that it was stable at pH 6[21,22]. Moreover, the stability of the complexes at pH 6, 7.4 and 8.6 has been investigated by electrophoresis on cellulose acetate strips according to the Chemetron Technique[23]. The electrophoretic mobility of the two proteins is different; under our experimental conditions, α2-M migrated to the anode, since HPE did not move and the complexes moved like α2-M.

Whether purified as above or used directly, the complexes were allowed to react with human aortic elastin[11]. The radioactivity of elastase allowed us to check that the complexes, or the enzyme when used alone, bound to elastin. The resulting elastinolytic activity was measured after the reactants had been equilibrated in adequate buffer (pH 8.6) and stirred for 1 h at 37 °C.

Results. Since the elastinolytic reaction takes place in heterogeneous phases, it was necessary, as a first step, to determine the optimum concentration conditions for all reactants. When a given amount of elastase (50 nM) was reacted with varying quantities of aortic elastin, the rate of elastinolysis became independent of substrate concentration up to 10 mg/ml elastin (fig. 1). Then, it was possible to investigate the rate of peptide release by elastase, when it was inhibited or not by increasing amounts of α2-M (fig. 2). Using the data from the slope of the curves, i.e. the remaining activity of elastase, it can be seen (fig. 2) that two moles of α2-M were necessary to inhibit completely one mole of elastase.

As shown in the table, human pancreatic elastase was able to form a stable complex when incubated with α2-M, since the radioactivity of elastase labeled with ^{125}Iodine was retained on Concanavalin A-Sepharose, with a wide variety of I/E ratios. Actually, pancreatic elastase is not a glycoprotein while the seric inhibitors α1-AP and α2-M are glycoproteins, thus the formed complex conserved its glycoprotein character. For this reason, the radioactivity of elastase complexed to α2-M remained bound to the Concanavalin A-Sepharose. Moreover, it should be noticed that the level of radioactivity increased with increasing amounts of labeled elastase. This may indicate that, besides the binding at the active site, the enzyme is able to link α2-M unspecifically. This important as well as unexpected fact may be

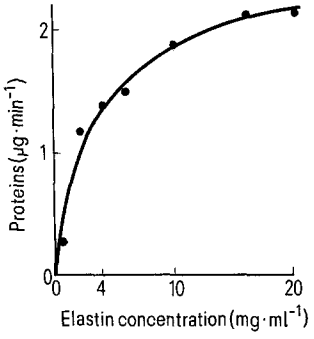

Figure 1. The rate of elastinolysis versus elastin concentration is expressed as the initial velocity of the reaction.

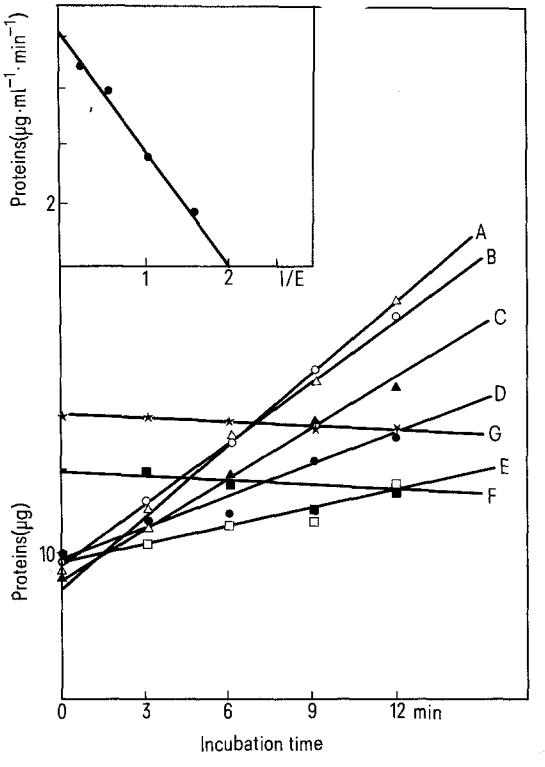

Figure 2. Kinetic curves of elastinolysis with increasing amounts of α2-M as described in the experimental section. A) I/E = 0 △—△—△; B) I/E = 0.2 ○—○—○; C) I/E = 0.5 ▲—▲—▲; D) I/E = 1 ●—●—●; E) I/E = 1.5 □—□—□; F) I/E = 2 ■—■—■; G) I/E = 3 *—*—*. On the upper part of the figure, the velocity of the remaining elastinolysis is expressed as a direct function of the I/E ratio.

due to the size of α2-M (725,000 mol.wt) and to the aggressive catabolic property of elastase. Even though the complexes were eluted with a low yield, a good purification of the complex was obtained as shown in line 2 of the table. When the complex between α2-M and HPE_{II} was equilibrated with factors promoting elastinolysis, the complex did not develop an elastinolytic activity until the I/E ratio was lower than 2, as shown in line 3 of the table. The electrophoretic study on cellulose acetate strips revealed that the complexes were very stable when the pH changed from 7.4 to 6 and 8.6. Moreover for I/E = 2 and 0.1 all the radioactivity of ^{125}I-elastase was found linked to α2-M (table), which corroborates the possibility of unspecific binding of elastase to α2-M.

As we have done before for α1-AP-HPE[11], it has been verified that the α2-M-HPE complex could be bound to elastin at pH 6

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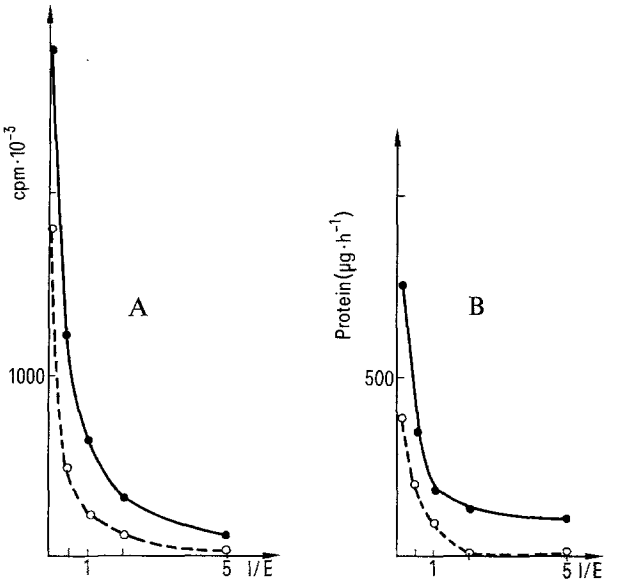
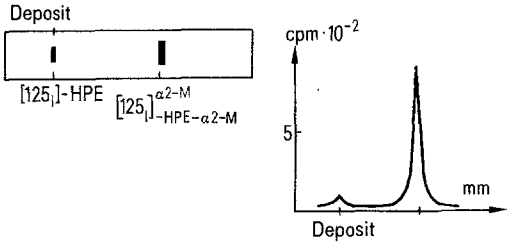


Figure 3. Binding on elastin – A – and subsequent elastinolysis – B – of free elastase (●—●—●) and elastase complexed to α2-M (○—○—○) at different I/E ratios (0.1, 0.5, 1, 2,5). From I/E < 2 the complexes develop properties comparable to free enzyme regarding binding and elastinolysis. Free elastase was at the same concentration as in the complex for each I/E value.

Formation, purification on Con A-Sepharose and characterization of the complex α2-M-HPE in relation to the respective amounts of both reactants. On the lower part, electrophoresis on cellulose acetate strip of the complex of α2-M with a large excess of ^{125}I-HPE (I/E ⩾ 0.1). All the radioactivity due to elastase migrated with α2-M.

α2, M / 125, I-HPE =	10	7	5	3	2	1	0,5	0,1
Radioactivity retained on ConA-Sepharose CPM, 10^{-3}	76	110	160	260	385	810	1500	3400
Purification of α2, M–HPE from the ConA-Sepharose CPM, 10^{-3}	5,7	10,4	15	23	38	93	190	600
Elastinolytic activity of the purified complexes Prot µg/h	0	0	0	0	0	20	40	150



(fig. 3). Obviously, we have verified that the change of buffer from pH 6 to pH 8.6 induced elastinolysis but only when I/E ratio was less than 2.

Discussion. α2-M, like α1-AP, is not a specific inhibitor of a given protease and inhibits a great number of proteolytic enzymes. In most cases this inhibition has been reported as non-reversible, from a thermodynamic point of view. Thus, the enzyme inhibited by α2-M loses its elastinolytic but retains its amidolytic activity[1,9]. Because of its non-reversibility, it is possible to determine the stoichiometric ratio I/E, and several conflicting results have been published. In these reports, the homology of substrates, enzymes and inhibitors, their degree of purification as well as the inhibition conditions are not always clearly described. Under our experimental conditions, two moles of α2-M are necessary to inhibit one mole of elastase. This surprising result cannot be related to the stoichiometry of previously described inhibition of proteolytic enzymes. In the case of the complex between porcine pancreatic elastase with homologous α2-M[7], a 1/1 stoichiometry was found but hemoglobin was used as substrate. Barret and Starkey[6] found the same ratio, but for trypsin. In the other hand, Bieth et al.[5] as well as Gustavsson et al.[9] more recently, gave a I/E ratio of 1/2; these authors determined the remaining activity, after the complex had been formed, with a casein-elastin substrate or using an immunoelectrophoresis technique.

The main interest of our work is that the three reactants used came from the same species, human, and were allowed to react simultaneously. The affinities between enzyme, substrate and inhibitor should be comparable to those in vivo. Our experimental conditions may explain why the stoichiometry is different from that published by others. However, when the complex was prepared before the incubation with elastin, the results were quite surprising since a minimum of 10 moles of enzyme were found to bind to one mole of α2-M (table), as is shown either by the amount of labeled elastase linked to α2-M or by electrophoresis on cellulose acetate. But the resulting complexes developed an elastinolytic activity only for an I/E ratio less than 2; the same result as with initial velocity determination. Because of the homology of the system we have used, the specificities between substrate, enzyme and inhibitor should be very close to the biological reality. Thus, the ratio of two moles of α2-M per mole of enzyme could be interpreted as a lower affinity or a modulated affinity implicating a reversibility of this reaction. The study of the inhibition of the elastin-elastase system by human plasma that contains both inhibitors α1-AP and α2-M seems to corroborate this reversibility. Actually, as has been previously shown by Meyer et al.[24], the elastinolytic activity was not completely destroyed in spite of increasing amounts of inhibitors. On the contrary, a marked activity was noticed. An exhaustive kinetic study is in process in our laboratory to determine the mechanism of elastinolytic inhibition by α1-AP and α2-M in human.

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DNA synthesis in exocrine and endocrine pancreas after partial hepatectomy in Syrian golden hamsters[1]

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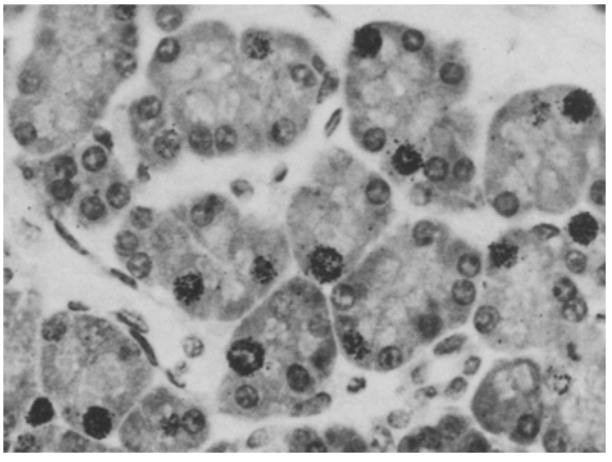
Summary. ^{3}H-thymidine autoradiography showed an enhanced DNA synthesis in acinar and islet cells of pancreas after partial hepatectomy in syrian golden hamsters. A significant nuclear labeling index of acinar cells was observed between 48 and 84 h and reached control levels by 120 h. An increased labeling index of islet cells was also observed, however, this increase was not statistically significant. These results indicate growth factor(s) produced after partial hepatectomy is capable of inducing DNA synthesis in pancreas.
Key words. Partial hepatectomy; DNA synthesis; thymidine; acinar cells.

Surgical removal of a portion of the liver results in compensatory hyperplasia of the remaining portion and reaches original weight within a few days in many species of animals[3]. During a study analyzing the effect of partial hepatectomy (PH) on pancreatic hepatocytes in hamsters[4], we have observed hyperplasia of pancreatic acinar cells. This finding was rather surprising because 1) the common notion is that the exocrine pancreas is a 'nondividing tissue'[5] and 2) pancreatic regeneration could be induced in adult hamsters, rats and guinea pigs only after producing pancreatic necrosis[6–9], or after surgical removal of a portion of the pancreas[10,11]. In the present experiment, we have systematically studied the effect of PH on stimulation of pancreatic DNA synthesis at different intervals. The results of this study demonstrate that PH leads to enhanced DNA synthesis in acinar and islet cells of the pancreas.

Methods. Seventy-five male syrian golden hamsters weighing 50–60 g were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). Hamsters were housed in plastic cages in groups of 3–4/cage on san-i-cel bedding under standard conditions of temperature, humidity and light dark cycle. All the hamsters were maintained on pelleted hamster diet (Tekland Test diets, Madison, Wis.) and had free access to water. In 42 hamsters partial hepatectomy (PH) was done by removal of median and left lobes as described before[4]. To avoid diurnal variation, PH was performed between 07.00 and 11.00 h. Hamsters were sacrified in groups of 3–4 at 6-h intervals, starting from 24 h after PH up to 72 h, and at 84, 96 and 120 h. Another 24 hamsters were subjected to sham operation and sacrificed in groups of 3–4 at 42, 48, 60, 72, 84, 96 and 120 h. The remaining 4 animals were used as standard controls. All the hamsters were given ^{3}H-thymidine i.p. (SP activity 2 ci/m mol; Research Product International Corp., Elk Grove Village, III.) at a dose of 1 μCi/g b.wt, 2 h before sacrifice. Portions of pancreas and liver were fixed in neutral buffered formalin and processed for light microscopy. Five micron thick paraffin sections were routinely stained with hematoxylin and eosin. To assess DNA synthesis, paraffin sections were coated with Kodak NTB_2 nuclear emulsion (Eastman Kodak Company, Rochester, NY) for autoradiography, as previously described[8]. Labeling index was obtained by counting 1000 nuclei from acinar and islet cells.

Results and discussion. Regenerative changes in the liver after PH are very similar to those previously published[4]. Pancreas of hepatectomized and sham operated animals were grossly unremarkable. Histologically, no necrosis or inflammation was observed. Labeling indices of acinar and islet cells in hepatectomized, sham operated and control animals are presented in the table. Increased incorporation of labeled thymidine into acinar cell nuclei was first detected at 42 h after PH, and reached a maximum at 54 h ($p < 0.001$) and persisted at high levels up to 84 h when compared with controls (fig.). A slight increase in label-



^{3}H-thymidine autoradiograph of hamster pancreas, 60 h after partial hepatectomy. Several acinar cell nuclei are labeled. Hematoxylin and eosin stain, × 550.