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Radioimmunoassay of Human Pepsinogen A and Pepsinogen C[1])

By *I. Biemond, J. B. M. J. Jansen, L. F. S. J. Crobach, Johanna Kreuning* and *C. B. H. W. Lamers*

Afdeling Maag-, Darm- en Leverziekten, Academisch Ziekenhuis, Leiden, The Netherlands

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Summary: We describe the development of radioimmunoassays to measure both human pepsinogen A and pepsinogen C concentrations in serum. The antibodies were raised in goats by immunization with purified pepsinogen A or C. The affinity constants of the respective antibodies were $20 \cdot 10^{10}$ l/mol and $7 \cdot 10^{10}$ l/mol.

Pepsinogens A and C were labeled with Na ^{125}I by the chloramine T method. The binding between labels and antibodies was inhibited by 0.50 at 0.82 ng pepsinogen A per tube and 2.1 ng pepsinogen C per tube. The detection limits of the assay of pepsinogen A and C were 0.12 μg/l and 1.8 μg/l, respectively.

Pepsinogen A and C were purified and added to a patient serum, showing a good recovery in the radioimmunoassays. Serial dilution of another patient serum, which contained a high concentration of both antigens, showed curves parallel to the standard curves. The intra- and interassay variations of these radioimmunoassays were evaluated. The intra-assay coefficients of variation for pepsinogen A were found to vary from 0.03 to 0.102 at concentrations in serum in the normal range, while the inter-assay coefficient of variation ranged from 0.118 to 0.194 at the same concentrations in serum. For the pepsinogen C radioimmunoassay we found intra-assay coefficients of variation between 0.126 and 0.147 at concentrations in serum in the normal range, while the inter-assay coefficient of variation ranged from 0.174 to 0.325 for the same sera.

In 201 blood donors we found a mean serum concentration of pepsinogen A of 59 μg/l and a mean serum concentration of pepsinogen C of 15 μg/l. There was a significant relationship between these values ($r = 0.779$, $p < 0.001$).

The concentrations of both pepsinogens were increased in 10 patients with duodenal ulcer (132 and 67 μg/l, respectively). Serum pepsinogen A was decreased in 14 patients with partial gastrectomy (34 μg/l) and in 6 with achlorhydria (9 μg/l), accompanied by raised (34 μg/l) and normal (20 μg/l) mean pepsinogen C concentrations in the respective groups. In 4 patients with a total gastrectomy the mean serum concentration of both pepsinogen A and C reached the zero level (5 and 4 μg/l, respectively). These results not only underline the accuracy of the measurements but also point to their clinical relevance.

Introduction

Pepsinogen A and C are the proenzymes of pepsin A and pepsin C, respectively, which belong to the group of gastric acid aspartic endoproteinases. Pepsinogen A is secreted by fundic mucosa, while pepsinogen C is secreted by fundic, pyloric and proximal duodenal mucosa (1). Exocrine-secreted pepsinogens function as zymogens for the main proteolytic activity of gastric juice. There is also a small endocrine excretion of pepsinogens without a known function, but by exploiting this phenomenon, we can gain information on the condition of the gastric mucosa by measurement of the serum concentration of pepsinogen A and pepsinogen C (2, 3).

[1]) Enzymes:
Pepsin A; EC 3.4.23.1
Pepsin C; gastricsin; EC 3.4.23.3

As the concentration of both zymogens in serum is low, we developed a radioimmunoassay to measure pepsinogen A and C in serum. This report describes a protocol for the isolation of pepsinogen A and C from gastric mucosa, the production of antibodies against these antigens in goats, the conditions of the radioimmunoassay, and the distribution of serum pepsinogen A and C in blood transfusion donors and in patients with duodenal ulcer, atrophic gastritis, and partial or total gastrectomy.

Materials and Methods

Reagents

Na ^{125}I was purchased from New England Nuclear, Boston, MA, USA; diethylaminoethyl(DEAE)-Cellulose DE-52 from Whatman. Maidstone, England, UK; Sephadex® G 100, PD-10® columns, Mono Q® (high pressure resistant monodisperse anion exchanger), and decanting suspension 2 (Micro-Sepharose® beads coupled with horse anti-sheep/goat immunoglobulin antibodies) from Pharmacia, Uppsala, Sweden; cellulose dialysis membrane, disodium ethylene-diaminetetraacetic acid (EDTA), bis[2-hydroxyethyl]imino-tris-[hydroxymethyl]-methane(BIS-TRIS), and bovine haemoglobin from Sigma, St. Louis, MO, USA; all other reagents from E. Merck, Darmstadt, F. R. G. Nonimmune horse serum was obtained from a local abattoir.

Equipment

Radioactivity was measured by an automatic gamma-scintillation counter, the Clinigamma 1272, with four 1.5 inch NaI crystals from Wallac Oy, Turku, Finland. High pressure anion exchange chromatography was performed with the FPLC® system from Pharmacia, Uppsala, Sweden.

Gastric mucosa

The stomach of a patient with a *Zollinger-Ellison* syndrome was cut open along the minor curvature. The mucosa from the corpus was stripped off the muscle layer, divided in parts and frozen.

Isolation of pepsinogen A and pepsinogen C

After thawing, the mucosa was homogenized in 0.02 mol/l phosphate buffer, pH 7.5 (3 ml/g of tissue) with a Sorvall® Omnimixer at maximum speed for 3 minutes on ice. The homogenate was centrifuged at 36500 *g* at 4 °C for 15 minutes. The supernatant was decanted and once more centrifuged at 100000 *g* at 4 °C for 60 minutes. After decanting, the supernatant was dialysed overnight against the same buffer.

The retentate was applied to the top of a column (2.6 × 40 cm) packed with DEAE-Cellulose to a bed height of 17 cm and equilibrated with the above described phosphate buffer. The column was washed with 125 ml 0.1 mol/l NaCl in the equilibration buffer. The pepsinogens were eluted in a single peak by a linear gradient of 200 ml ranging from 0.10 mol/l to 0.48 mol/l NaCl in the equilibration buffer. The eluate fractions containing most proteolytic activity were pooled and dialysed against phosphate buffer.

The pooled pepsinogens were further purified by gel permeation chromatography on a 2.6 × 100 cm column with a flow adaptor packed with Sephadex® G 100 to a bed height of 93 cm in phosphate buffer at 4 °C.

All material from the Sephadex G 100 absorbing at 280 nm, was adsorbed to Mono Q® anion exchanger, then separated into pepsinogen C and pepsinogen A by FPLC® (fig. 1). The peaks of pepsinogen A were pooled and apart from the pepsinogen C peak thoroughly dialysed against distilled water and lyophilized.

Analytical methods

Proteolytic activity was monitored in column eluate fractions by incubating 0.1 ml of an appropriate dilution of sample with 2.5 ml bovine haemoglobin solution (2.5 g/l bovine haemoglobin in 0.06 mol/l HCl) at 37 °C for 10 min. The reaction was stopped with 5.0 ml of 0.31 mol/l trichloroacetic acid. The digest was filtered, and the absorbance of the filtrate was determined at 280 nm.

FPLC® was performed according to *Défize* et al. (4). Pepsinogens in phosphate buffer were applied to a 0.5 × 5 cm column, packed with Mono Q®, and equilibrated in 0.025 mol/l BIS-TRIS/hydrochloric acid, pH 6.0. The column was washed with 4 ml 0.12 mol/l NaCl in equilibration buffer, and the pepsinogens were eluted with 21 ml of a linear gradient of NaCl in equilibration buffer, ranging from 0.12 to 0.4 mol/l. Fractions of 0.5 ml were collected. Absorbance at 280 nm was continuously measured in the eluate.

Antibody production

Antibodies to pepsinogen A and pepsinogen C were raised in two different goats. Primary immunization was done by subcutaneous injection of an emulsion of 1 ml *Freund's* complete adjuvant and 1 ml solution of antigen in saline (1 g/l). The animals were boosted by monthly injections of an emulsion of 1 ml *Freund's* incomplete adjuvant and 1 ml solution of antigen in saline (0.5 g/l), and bled at two week intervals. The injection scheme was continued for more than two years.

Dilution curves of goat anti-pepsinogen A for 4 different bleeding times were compared and the best selected. A goat anti-pepsinogen C antiserum, taken at a randomly chosen bleeding time, showed an appropriate dilution curve.

Radioiodination

Pepsinogens A and C were labeled with Na ^{125}I by the chloramine T method (5). The incubation mixture contained 5 µg pepsinogen A or pepsinogen C in 5 µl 0.05 mol/l phosphate buffer, pH 7.5, 20 µl 0.5 mol/l phosphate buffer, pH 7.5, 5 µl Na ^{125}I solution (3700 GBq/l) and 10 µg (10 µl) chloramine T. The mixture was incubated for 30 s at 0 °C. The reaction was stopped by addition of 20 µg (10 µl) sodium metabisulphite and diluted with 1.0 ml 0.05 mol/l phosphate buffer, pH 7.5.

Labeled pepsinogens and inorganic ^{125}I were separated on a PD-10® column (15.2 × 50 mm) which was equilibrated and eluted with 0.05 mol/l phosphate buffer, pH 7.5. Eluate fractions of 1.0 ml were collected into tubes containing 10 µl horse serum. The fraction next to the void volume fraction was used and diluted in 0.01 mol/l phosphate-buffered saline containing 10 ml/l horse serum.

Radioimmunoassays

Pepsinogen A and pepsinogen C were both measured by a competitive binding assay in which bound and free antigen were separated by Micro-Sepharose® beads coupled to horse anti sheep/goat immunoglobulin antibodies. The diluent buffer was a mixture of 6 volumes 0.01 mol/l phosphate buffered saline, pH 7.5, and one volume 0.1 mol/l EDTA in water,

supplemented with 10 ml/l nonimmune horse serum. Diluent buffer, antiserum dilution, standards or test sera were added to 11.5 × 55 mm disposable polystyrene tubes to a final volume of 1 ml.

For the radioimmunoassay of pepsinogen A the incubation mixture contained 400 µl diluent buffer, 200 µl pepsinogen A standard or 10-fold diluted test serum, 200 µl goat anti-pepsinogen A in a final dilution of 1 : 300 000, and 200 µl ^{125}I-labelled pepsinogen A in diluent buffer to provide about 4000 counts/min (120 Bq per tube).

For the radioimmunoassay of pepsinogen C the same scheme was applied but ^{125}I-labelled pepsinogen C was used as label, goat anti-pepsinogen C was used in a final dilution of 1 : 200 000, and the test sera were used in a 5-fold dilution in buffer.

After incubation for 96 h at 4 °C, 200 µl decanting suspension containing Micro-Sepharose® beads coupled with horse anti-sheep/goat immunoglobulin antibodies was dispensed, stirred, and incubated at room temperature for 2 hours. The tubes were then centrifuged at 4500 *g* for 10 min, the supernatant was decanted and the radioactivity of the pellet was counted for 5 min in a automatic gamma counter.

Standards were prepared by dissolving a weighed quantity of lyophilized pepsinogen A or C in diluent buffer. All standards and test sera were set up in duplicate. The bound radioactivity was corrected for nonspecific binding by subtracting the mean radioactivity in the nonspecific binding tubes from that in the standards and unknowns. Standard curves were calculated by linear regression of the logit transformed B/B_0 ratio with the logarithm of the standard concentration. The affinities of the antisera were calculated according to *Scatchard* (6).

Five control sera were used for measurement of the intra- and inter-assay variation, containing 3, 27, 39, 81, and 317 µg/l pepsinogen A and 10, 22, 8, 12, and 523 µg/l pepsinogen C, respectively.

Test sera

Serum was obtained from 201 different blood transfusion donors, 130 males and 71 females, ranging in age between 18 and 64 years; from 10 patients with duodenal ulcer (8 ♂, 2 ♀; age 21–63 years); from 14 patients who had undergone a partial gastrectomy (12 ♂, 2 ♀; age 27–71 years) of which 7 had a gastroduodenostomy and the others a gastrojejunostomy; from 4 patients with a total gastrectomy (3 ♂, 1 ♀; age 63–84 years); and from 6 patients with achlorhydria due to atrophic gastritis (4 ♂, 2 ♀; age 53–78 years).

Statistical analysis

Student's t test using a pooled or separate variance estimate, depending on the homogeneity of both variance, was applied to evaluate the significance of differences between the means of two groups. The significance of differences between the means of more than two groups was evaluated by the ratio of variances within and between groups. The level of significance was taken at 0.05.

Results

Purification and characterization

FPLC® anion exchange chromatography of the partially purified pepsinogens on Mono Q® produced 4 major peaks of absorbance at 280 nm, all of which showed proteolytic activity (fig. 1). The ratio between proteolytic activities was 2.2 for the first peak and 0.8 for the 3 following peaks. The first peak, further referred to as pepsinogen C, was immunologically distinguishable from the pooled 3 other peaks, further referred to as pepsinogen A; differentiation was possible with the aid of goat anti-pepsinogen C, which did not bind to radiolabeled pepsinogen A, and goat anti-pepsinogen A, which did not react with radioiodinated pepsinogen C. Furthermore, pepsinogen C showed no inhibition of the binding of goat anti-pepsinogen A and labeled pepsinogen A, and pepsinogen A showed no inhibition of the binding between goat anti-pepsinogen C and radiolabeled pepsinogen C (fig. 2).

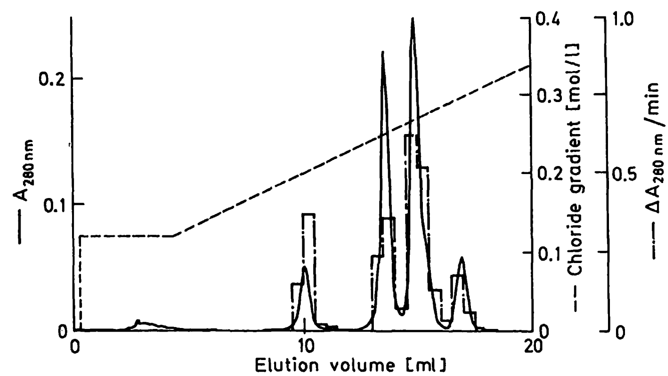

Fig. 1. FPLC®-gel permeation chromatography of gastric acid proteases on Mono Q® anion exchanger, using a chloride gradient. Solid line: absorbance at 280 nm; dashed-dotted line: absorbance increment at 280 nm per min during incubation of filtrate from bovine haemoglobin incubated with eluted fraction; dashed line: chloride gradient. The first peak eluted at 10 ml represents pepsinogen C, while the following 3 peaks represent isozymogens of pepsinogen A.

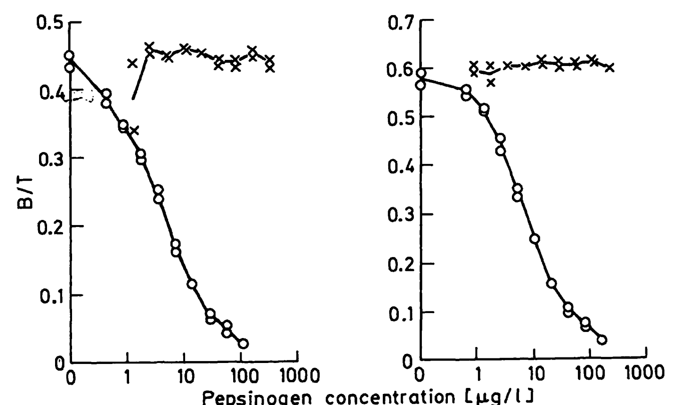

Fig. 2. Left: Ratio of bound fraction to total radioactivity versus decadic logarithm of concentration using radiolabeled pepsinogen A and goat anti-pepsinogen A. Pepsinogen A (o) binds to antibody, but not to pepsinogen C (x).
Right: Ratio of bound fraction to total radioactivity versus decadic logarithm of concentration using radiolabeled pepsinogen C and goat anti-pepsinogen C. Pepsinogen C (o) binds to antibody, but not to pepsinogen A (x).

Radioimmunoassay of pepsinogen A

Radiolabeled pepsinogen A was found to have a specific activity of 0.318 MBq/µg. Thus each tube contained about 0.5 ng radiolabeled pepsinogen A. About 0.45 of radioactivity was bound by goat anti-pepsinogen A serum in a final dilution of 1 : 300 000. The nonspecific binding was about 0.01 – 0.02. The equilibrium constant was found to be $20 \cdot 10^{10}$ l/mol under the conditions of the assay. The detection limit, corresponding to two standard deviations less than the mean counts bound in 5 zero standards in duplicate, was 0.12 µg/l. The 0.50 inhibitory dose was about 0.82 ng/tube. This high sensitivity allowed us to use the test sera in a 10-fold dilution.

A calibration curve in which the concentration of pepsinogen A standard ranged from 0.44 to 112 µg/l (0.09 to 22.5 ng/tube) is shown in figure 2. Pepsinogen C to a concentration of 340 µg/l did not compete in the assay (fig. 2), and goat anti-pepsinogen C in a final dilution of 1 : 200 000, did not bind radiolabeled pepsinogen A.

The recovery of pepsinogen A from a serum spiked with pepsinogen A standard in concentrations ranging from 0.44 to 112 µg/l varied between 0.813 and 1.297 with a median of 1.032 (fig. 3). A control serum containing a high concentration of pepsinogen A and C was serially 2-fold diluted up to 1 : 512 and showed a curve parallel to the standard curve (fig. 3). The intra-assay coefficients of variation for pepsinogen A were found to vary from 0.030 to 0.102 at concentrations in serum in the normal range, while the inter-assay coefficient of variation, determined over a one-month period, ranged from 0.118 to 0.194 at the same serum concentrations (fig. 4).

Radioimmunoassay of pepsinogen C

Radiolabeled pepsinogen C had a specific activity of 0.651 MBq/µg. Thus each tube contained about 0.18 ng radioiodinated pepsinogen C. About 0.55 of radioactivity was bound by goat anti-pepsinogen C serum in a final dilution of 1 : 200 000. The nonspecific binding was about 0.01 – 0.02. The equilibrium constant was found to be $7 \cdot 10^{10}$ l/mol under the conditions of the assay. The detection limit, corresponding to two standard deviations less than the mean counts bound in 5 zero standards in duplicate, was 1.8 µg/l. The 0.50 inhibitory dose was about 2.1 ng/tube. This sensitivity allowed us to use the test sera in a 5-fold dilution.

A calibration curve in which the concentration of pepsinogen C standard ranged from 0.66 to 169 µg/l (0.13 to 34 ng per tube) is shown in figure 2. Pepsinogen A to a concentration of 225 µg/l did not compete in the assay (fig. 2), and goat anti-pepsinogen A in a final dilution of 1:300 000 did not bind radiolabeled pepsinogen C.

The recovery of pepsinogen C from serum spiked with pepsinogen C standard in concentrations ranging from 2.64 to 169 µg/l varied between 0.859 and 1.395 with a median of 1.159 (fig. 2). A control serum containing a high concentration of pepsinogen A and C was serially 2-fold diluted up to 1 : 128 and showed a curve parallel to the standard curve (fig. 3). For the pepsinogen C radioimmunoassay we found an intra-assay coefficient of variation between 0.126 and 0.147 at serum levels in the normal range, while the inter-assay coefficient of variation, determined over a one-month period, ranged from 0.174 to 0.325 for the same sera (fig. 4).

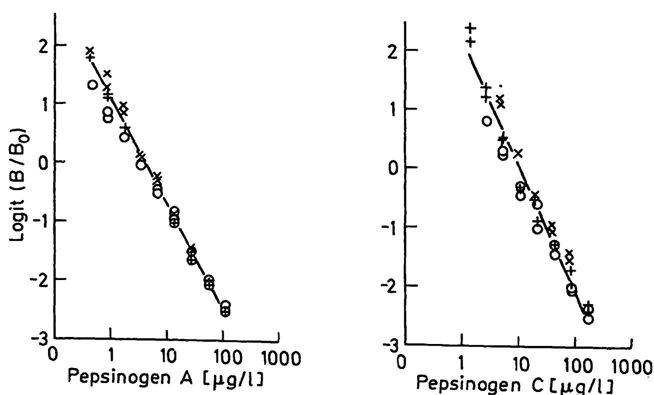

Fig. 3. Standard curve (+) of logit transformed B/B_0 versus decadic logarithm of concentration using radiolabeled pepsinogen A (left) or pepsinogen C (right) and goat anti-pepsinogen A (left) or goat anti-pepsinogen C (right).
Recovery (o) of lyophilized pepsinogen A (left) or pepsinogen C (right) added to a human serum.
Dilution curve (x) of patient serum containing a high concentration of both pepsinogen A and C.

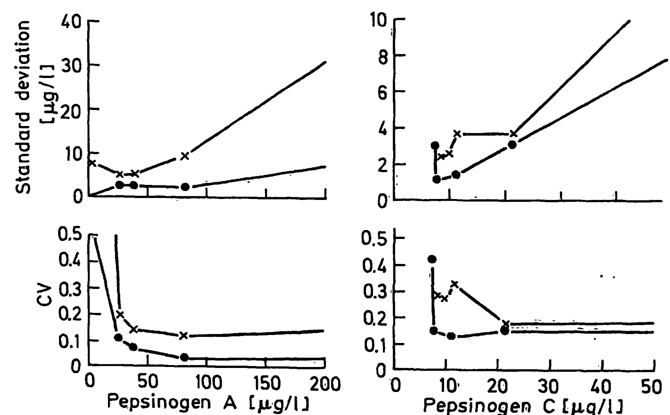

Fig. 4. Intra-assay (●—●) and interassay (x—x) variation expressed as standard deviation (SD) or coefficient of variation (CV) of pepsinogen A (left) and pepsinogen C (right) in 6 duplicate determinations.
CV is given as the quotient $CV = \frac{SD}{\bar{x}}$.

Tab. 1. Means ($\bar{x}$) and standard deviations (SD) of the concentrations of serum pepsinogens A and C (μg/l) and the ratio of pepsinogen A and pepsinogen C in blood transfusion donors and different patient groups.

	n	Pepsinogen A		Pepsinogen C		Ratio	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Blood transfusion donors	201	59	28	15	12	5.2	3.1
– males	130	65†	29	16	12	5.1	2.4
– females	71	48	22	13	11	5.4	4.1
Duodenal ulcer disease	10	132*	57	67*	25	2.1*	0.8
Partial gastrectomy	14	34*	29	34*	31	4.4	7.4
– gastroduodenostomy	7	28*	15	26*	18	4.7	9.4
– gastrojejunostomy	7	41	39	42	40	2.5*	4.2
Total gastrectomy	4	5*	4	4*	4	0.5*	0.5
Atrophic gastritis	6	9*	8	20	17	0.8*	0.7

† significant different between males and females
* significant different from blood donors

Pepsinogen A and C concentrations in test sera

The mean serum concentration (± SD) of pepsinogen A in blood transfusion donors was 59 (± 28) μg/l (tab. 1). The serum concentration of pepsinogen A showed a leptokurtic and positively skewed distribution, which could be nearly normalized by square-root transformation. In males the mean concentration of pepsinogen A in serum was 65 (± 29) μg/l and in females a mean concentration of 48 (± 22) μg/l was found; this difference was statistically significant (tab. 1).

Pepsinogen C was found to be 15 (± 12) μg/l for all blood transfusion donors (tab. 1). The serum concentration of pepsinogen C showed a leptokurtic and positively skewed distribution, which could be partly normalized by square-root transformation. In contrast to pepsinogen A (tab. 1), the mean concentration in males (16 ± 12 μg/l) was not statistically different from that in females (13 ± 11 μg/l).

Serum pepsinogen A, C and its ratio in relation to the age of the blood transfusion donors showed a gradual increase of both pepsinogen A and C with increasing age of the donors. The ratio tended to decrease in older people (tab. 2).

Patients with duodenal ulcer showed an increase of serum pepsinogens A and C, while the ratio decreased, due to the relatively strong increase of pepsinogen C. Patients having undergone partial gastrectomy as well as patients with achlorhydria showed decreased levels of pepsinogen A, but equal or even increased levels of pepsinogen C. The ratio also tended to decrease, and atrophic gastritis patients had an especially low ratio. Total gastrectomy caused a strong decrease in the serum concentrations of both pepsinogens and their ratio (tab. 1).

In figure 5 the pepsinogen A concentration in serum was plotted against pepsinogen C in both patients and blood donors. There was a highly significant overall correlation between both concentrations ($r = 0.671$, $p < 0.001$). For blood donors and different groups of patients the correlation was found to be equally strong, except for patients with duodenal ulcer, for which the correlation coefficient was found to be much lower. The blood donors and each group of patients are characterized by their own intercept and slope of the regression line.

Tab. 2. Means and standard deviations of the concentrations of serum pepsinogens A and C (μg/l) and the ratio of pepsinogen A and pepsinogen C in different age groups of blood transfusion donors.

Age group (a)	n	Pepsinogen A*		Pepsinogen C*		Ratio	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
20–29	60	50	20	12	7	5.6	4.1
30–39	60	61	29	15	13	5.1	2.2
40–49	53	63	27	17	11	5.0	3.1
50–59	21	73	34	19	16	5.2	2.4
60–64	6	69	37	23	6	3.0	1.4

* significant ratio of within and between variances

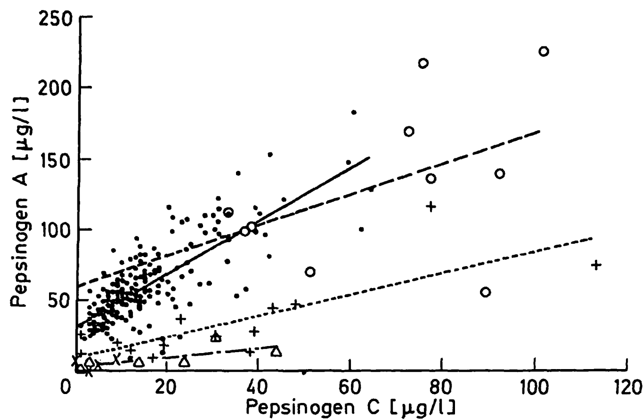

Fig. 5. Relationship between serum pepsinogen A and serum pepsinogen C in blood donors and different groups of patients. Solid line: regression line ($r = 0.779$; $y = 1.88x + 30.87$) in blood donors (●). Dashed line: regression line ($r = 0.473$; $y = 1.09x + 60.23$) in patients with duodenal ulcer (○). Dotted line: regression line ($r = 0.790$; $y = 0.75x + 9.10$) in patients having undergone partial gastrectomy (+). Dotted-dashed line: regression line ($r = 0.706$; $y = 0.33x + 2.76$) in patients with atrophic gastritis (△). Patients with total gastrectomy (×).

Discussion

We describe the purification and radioimmunoassays of pepsinogens A and C and their serum concentration distribution in blood transfusion donors, in patients suffering from duodenal ulcer disease, in those having undergone partial or total gastrectomy, and in patients with achlorhydria.

With the purification of pepsinogens we followed partially the procedure described by *Samloff* (7). However for the separation of pepsinogen A from pepsinogen C we used anion exchange chromatography on Mono Q® by FPLC®. With a chloride gradient, pepsinogen C can be predicted to elute first from Mono Q®, in agreement with the order of elution of pepsinogens from Mono Q®- and DEAE anion exchangers by a chloride gradient as described by other authors (4, 7, 9). It has been shown in other studies (7, 10) that pepsinogen A is immunologically distinct from pepsinogen C; since the first peak appeared to be immunologically distinct from the following 3 peaks of the elution profile, we assigned the first peak in figure 1 to pepsinogen C and the others to pepsinogen A. The isozymogen profile of pepsinogen A from this study differs from that published before by *Défize* et al. (4), as these authors showed pepsinogen A to have 4 isozymogens. The reason for this discrepancy is probably genetically determined. It is known that the isozymogen profile of pepsinogen A is under genetic control (8), and the number of isozymogens of pepsinogen A and their intensity varies individually.

From figure 1 it is evident that the ratio between proteolytic activity and absorbance is larger for the pepsinogen C than for the pepsinogen A peaks. This finding has also been reported previously by *Samloff* (7).

In this study, the standards with the highest immunoreactivity were selected. For pepsinogen C in blood donors we found a mean value comparable to that of the controls published by *Samloff* (7); however, for pepsinogen A in the blood transfusion donors we found a mean value which was twice the mean concentration reported by *Samloff* (7). In order to reach uniformity we corrected our standard with a factor of two.

Because of differences in assay characteristics and the studied populations, any comparison of our study with those of others has to be interpreted with great caution. Nevertheless it is possible to compare trends in different patient and control groups from this study with those reported in the literature.

Both pepsinogens showed a leptokurtic and positively skewed distribution of their serum concentrations. Square root transformation reduced both the leptokurtosis and skewness, and this finding is in agreement with other studies (11, 12). In nearly all publications relating to the serum level of pepsinogen A in different age groups of control subjects, the same conclusion was reached as in this study, i. e. a gradual increase of pepsinogen A with age, reaching a maximum at the sixth or seventh decade of life (10, 12–16). We also found an increase of serum pepsinogen C in older healthy controls, as was also seen in other studies (9, 10).

The finding of a higher serum concentration of pepsinogen A in males than in females is in agreement with previous studies (12–17, 19). With respect to serum pepsinogen C, the picture is more complicated; some authors described a significantly higher serum concentration in males than in females (10, 18), while, in agreement with our findings, a more recent study found no significant difference (9).

In this study, both serum pepsinogens A and C were increased in duodenal ulcer disease, as was previously reported for serum pepsinogen A (10, 15, 16, 20, 21) and serum pepsinogen C (10, 20). In contrast to other studies (10, 20), we observed a significant reduction of the pepsinogen A to pepsinogen C ratio in these patients. The reason for this discrepancy is not apparent, but may be related to the relatively small number of patients studied in the present report.

In patients having undergone partial gastrectomy we found a decrease of pepsinogen A in serum, while

serum pepsinogen C showed an increase. This has also been reported by other authors (3, 15, 22) for pepsinogen A, while *Sipponen* et al. (3) reported that serum pepsinogen C increases in patients having undergone partial gastrectomy, as the atrophy of the mucosa aggravates.

Total gastrectomy caused a strong decrease in the serum concentrations of both pepsinogens, and their ratio as has been reported in other studies (7, 10).

Atrophic gastritis caused a decrease of serum pepsinogen A, while serum pepsinogen C was as high as in blood donors. These results fit perfectly with those published in the literature for pepsinogen A (2, 7, 17) and pepsinogen C (2, 7).

In summary, we have developed radioimmunoassays for both pepsinogen A and C in human serum, and we have applied these assays to measurements in healthy blood donors, and in patients with duodenal ulcer, partial or total gastrectomy and atrophic gastritis.

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Izak Biemond
Afdeling Maag-, Darm-, en Leverziekten
Gebouw 1, C4-P
Academisch Ziekenhuis
Postbus 9600
NL-2300 RC Leiden