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# CHROMATOGRAPHIC PATTERNS OF INDIVIDUAL GASTRIC JUICE SPECIMENS FROM NORMAL HUMAN SUBJECTS *

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Separation of the large molecular components of pooled human gastric juice into fractions of different biological activity by means of the Amberlite IRC-50 resin column has been reported earlier (1–6). In the present study a modification of this technic has been adapted to the fractionation of individual specimens of gastric juice. These modifications have improved and further standardized the older method.

## METHOD

Gastric juice was obtained from fasting healthy subjects through a nasogastric tube by means of constant hand suction (7). The subjects were encouraged to avoid swallowing saliva. The overnight residual and the material aspirated during the first 10 to 15 minutes were discarded. Basal secretion was then collected for 0.5 hour, after which collections were continued for an additional 75 minutes with or without stimulation by either histamine sulfate (0.01 mg per kg, s.c.) or regular insulin (10 U, i.v.). The samples were kept cold and filtered through Pyrex wool. The volume of each specimen was recorded in milliliters. The titratable acidity was determined against Topfer's reagent and phenolphthalein. The specimens obtained from a single individual prior to and after stimulation were then separately pooled, dialyzed at 4° C against five changes of distilled water for 48 hours, and lyophilized (2). The content of hexose (8) and total proteolytic activity (9) of the lyophilized material was determined. The hexoses were expressed in milligrams per 100 mg of nondialyzable lyophilized gastric solids. The proteolytic activity (proteolytic units, PU) was recorded in $PU_{Hb} \times 10^{-4}$ per mg of nondialyzable lyophilized gastric solids. Fifty mg of the dialyzed and lyophilized material was dissolved in 1 ml of a pH 3.0 buffer and kept for 24 hours at 4° C. The material was warmed to room temperature and loaded on an Amberlite IRC-50 resin column, $1 \times 11$ cm. Elution was achieved with 0.2 M sodium citrate buffers, from pH 3.0 to 4.6, and followed by 0.5 M phosphate buffers, from pH 5.3 to 7.5. The buffers were applied stepwise, without interruption, over a 30- to 36-hour period (Table I). Equal volumes (1 or 2 ml) of effluent were collected at a rate of approximately 2 ml per 11 minutes. The pH of the effluent was measured with a Beckman pH meter. The effluent in each tube was analyzed for protein (10), hexoses (8), and proteolytic activity (9). The determinations of proteolytic enzyme activity in the whole lyophilized gastric juice and in the collection tubes eluted from the column were made by the method of Glass, Pugh and Wolf (9) at a pH of 1.5. This, of course, is near the pH optimum of pepsin, but at this pH gastricsin has some degree of specific activity (4). Thus it is not possible to discriminate between the proteolytic enzyme activity in the whole gastric juice derived from pepsin or gastricsin unless the two components are completely separated by fractionation. This has been accomplished and reported separately by Tang, Wolf, Caputto and Trucco (4). Nevertheless, by using the same technic to determine the proteolytic activity throughout, it was possible to make meaningful comparisons without specific inferences regarding relative pepsin or gastricsin concentration. The presence of blood group A or B substance was determined by the hemagglutination inhibition technic in subjects of the corresponding blood groups (11). The remaining eluate from the area of each protein peak was dialyzed in a cold room against six changes of distilled water for 72 hours with constant agitation in a mechanical stirrer. The material was then lyophilized and stored for further studies of other biological activities.

## RESULTS

When the eluted protein, expressed in absorbance (A) units at 750 mμ, was plotted against the effluent volume, there were six distinct and separate protein-containing peaks in the chromatograms from normal individuals, as shown in Figure 1. For purposes of comparison the area of each protein-containing peak was calculated by taking the summation of the products of the absorbance units and the volume in each collection tube; i.e., area $= \Sigma\,(\text{vol} \times A)$. The sum of the area of the peaks was taken as a

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TABLE I

*Buffer system*

| Buffer pH | Type | Time when buffer was added |
|---|---|---|
| 3.0 | 0.2 M sodium cit. | Initial buffer |
| 3.5 | 0.2 M sodium cit. | When 20 ml effluent collected |
| 4.15 | 0.2 M sodium cit. | When 40 ml total effluent collected |
| 4.6 | 0.2 M sodium cit. | When 75 ml total effluent collected |
| 5.3 | 0.5 M phosphate | Effluent pH 4.55 |
| 6.2 | 0.5 M phosphate | Effluent pH 5.15 |
| 7.5 | 0.5 M phosphate | Effluent pH 6.15 |

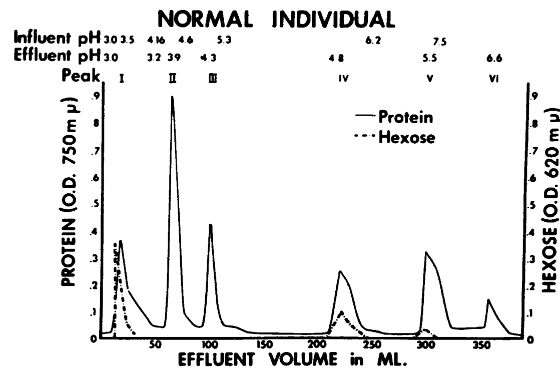


FIG. 1. CHROMATOGRAPHIC PATTERN OF A NORMAL INDIVIDUAL (T.P., 3). There are six protein peaks; peak II contains pepsin and peak III gastricsin; hexoses are eluted with protein peaks I, IV, and V.

measure of the total protein recovered. The hexoses eluted, expressed in A units at 620 mμ, were plotted against the effluent volume. These readings were converted to milligrams of hexoses for comparison.

*Reproducibility.* To measure the reproducibility of the technic, duplicate chromatograms were performed on three separate, individual samples of dialyzed and lyophilized juice. The data from such a pair of chromatograms are presented in Table II. The original gastric juice in this case contained 26 mEq per L of HCl. The proteolytic enzyme activity of the sample after it was dialyzed and lyophilized was 49.4 $PU_{Hb} \times 10^{-4}$ per mg and its hexose content was 10.6 mg per 100 mg. Two separate resin columns and fraction collectors were used. The effluent was collected in 1-ml volumes from one column and in 2-ml volumes from the other column. It will be noted in Table II that the two patterns are almost identical. Similarly, on the other two occasions, the method yielded satisfactory reproducibility.

*Blood group substance.* Using a larger Amberlite IRC-50 resin column, Richmond, Caputto and Wolf (2) had shown that blood group substance A was concentrated prior to the first ninhydrin peak eluted from the column and that the activity was not present in other fractions. To test whether the smaller column also had the ability to concentrate blood group substances, the gastric juice from six secretors was individually chromatographed (four type A and two type B). The redialyzed and lyophilized material from the individual eluted protein-containing peaks was assayed for the presence of blood group A or B substance by the hemagglutination inhibition method (11). Blood group substances A or B were found only in the material from protein peak I.

*Proteolytic activity.* Richmond, and Tang and their co-workers (3, 4), reported that the proteolytic activity in human gastric juice occurred in two fractions eluted from the resin column at pH 4.0 and 4.4, respectively. These workers were able to demonstrate that the first of the two contained pepsin and that the second contained an additional proteolytic enzyme with a higher pH optimum. Ultimately they purified this latter substance, crystallized it, and named it gastricsin (4). In the present study as well, proteolytic activity was found only in the area of two peaks—peak II containing pepsin and peak III, gastricsin. The proteolytic activity in

TABLE II

*Reproducibility: Data on two chromatograms of the same gastric juice*

| | Vol. effluent collected | Material eluted from the column | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Proteins | | | | | | | Hexoses | | | |
| | | Total, 6 peaks | Peak I area | Peak II area | Peak III area | Peak IV area | Peak V area | Peak VI area | Total | H-I | H-IV | H-V |
| | *ml* | | | | | | | | *mg* | *mg* | *mg* | *mg* |
| 1 | 1 | 29.86 | 7.33 | 9.52 | 4.24 | 3.70 | 3.70 | 1.37 | 3.07 | 1.89 | 0.87 | 0.31 |
| 2 | 2 | 29.67 | 7.14 | 9.38 | 4.49 | 3.81 | 3.49 | 1.36 | 3.17 | 2.00 | 0.85 | 0.32 |

TABLE III

*Comparison of protein and proteolytic activity of peaks II and III*

| $PU_{Hb} \times 10^{-4}$* peak II | Area† protein, peak II | $PU_{Hb} \times 10^{-4}$* peak III | Area† protein, peak III |
|---|---|---|---|
| 491 | 3.075 | 514 | 2.055 |
| 1,229 | 6.433 | 550 | 2.095 |
| 1,392 | 6.661 | 675 | 2.480 |
| 1,580 | 7.549 | 786 | 3.160 |
| 1,601 | 8.840 | 850 | 3.015 |
| 2,144 | 10.190 | 870 | 3.560 |
| 2,510 | 10.498 | 961 | 3.750 |
| 2,779 | 11.871 | 974 | 3.860 |
| 3,017 | 12.970 | 1,117 | 5.090 |
| 3,039 | 12.143 | 1,244 | 5.112 |

* Total of $PU_{Hb} \times 10^{-4}$ in collection tubes.
† Area = $\Sigma$ (vol $\times$ A).

each collection tube and the area of the protein-containing peaks II and III were measured in each of 10 chromatograms. These data are presented in Table III. There is a 1 : 1 relationship in the ranking of the area of the individual protein peaks and their content of proteolytic activity; i.e., if the protein peak areas are arranged from low to high the corresponding proteolytic activity values are arranged in the same order. To further substantiate this the product-moment correlation coefficients (12) were determined for the data in Table III. The r values for the area of peak II and its proteolytic activity, and the area of peak III and its proteolytic activity, were both 0.99. This indicates a probability under the null hypothesis (r not significantly different from zero) of $< 0.001$. Therefore, in comparing subsequent chromatograms, the areas of protein peaks II and III were taken as a rough index of the activity of the two enzymes.

*Normal patterns.* Sixteen chromatograms were performed on individual specimens obtained, with and without stimulation, from six medical students who did not have known gastrointestinal disease. The milliequivalents per liter (mEq/L) of HCl of the original gastric juice, the content of hexoses and total proteolytic enzyme activity of the nonfractionated dialyzed and lyophilized material, and the data from the eluted chromatograms are presented in Table IV. The gastric juice chromatograms of each of these healthy subjects were almost superimposable with respect to the location of their six protein-containing peaks. The protein peaks were eluted from each at the same effluent pH: peak I at pH 3.0; peak II at pH 3.9; peak III at pH 4.3; peak IV at pH 4.8; peak V at pH 5.45; and peak VI at pH 6.6. Hexoses appeared only in the areas of protein peaks I, IV, and V. The three hexose peaks are referred to as H-I, H-IV, and H-V. The first hexose peak, H-I, usually started simultaneously with the protein peak I, whereas the other two hexose peaks (H-IV and H-V) appeared slightly after the start of the protein peaks IV and V. Hexose peak H-IV was absent in one chromatogram (J.J., 3) and H-V was absent in another (T.P., 1). Protein peak V was notched in three chromatograms (T.P., 2; J.D.M., 1; and D.S., 1). In each case the bottom of the notch coincided with the end of the hexose peak H-V.

*Relation of pattern to acid secretion.* From inspection of the data in Table IV, certain general intraindividual variations in the chromatograms appeared to correlate with the concentration of hydrochloric acid in the original juice. As the mEq/L of HCl in the individual samples increased: *a*) the sum of the areas of the six protein-containing peaks increased; *b*) the areas of peak II and III, reflecting the activity of the proteolytic enzymes, increased; *c*) the total hexoses recovered (sum of hexose peak H-I, H-IV, and H-V in milligrams) in the eluate decreased; and *d*) the hexose in peak H-I decreased. Although only tentative inferences may be drawn from a statistical analysis of such data as these, the potential significance of intraindividual and interindividual relationships between the mEq/L of HCl in the original juice and the distribution of large molecular components in the chromatograms seemed to warrant the attempt. We therefore made an initial trial, using the technic of the product-moment correlation coefficient (12). From such a calculation r equaled + 0.606 which indicated a probability under the null hypothesis (r not significantly different from zero) of $< 0.01$. Accordingly, the possible correlation of the mEq/L of HCl of the original juice and the composition of the nondialyzable lyophilized material was tested. We found that there was a significant positive correlation of the free acid with the proteolytic enzyme activity of this material (r = + 0.675) while there was a negative correlation with its content of hexoses (r =

TABLE IV

*Data on the dialyzed and lyophilized gastric juice and the chromatograms of normal individuals*

| Patient | Stim.* | Free acid original juice | Dialyzed and lyophilized juice | | Material eluted from the column | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Proteins | | | | | | | Hexoses | | | |
| | | | Proteolytic activity (total) | Hexose | Total area of 6 peaks | Peak I area | Peak II area | Peak III area | Peak IV area | Peak V area | Peak VI area | Total | H-I | H-IV | H-V |
| | | mEq/L | $PU_{Hb} \times 10^{-4}$/mg | mg/100 mg | | | | | | | | mg | mg | mg | mg |
| T.P., 1 | N | 19 | 32.7 | 14.5 | 23.5 | 4.6 | 6.5 | 2.5 | 5.4 | 3.2 | 1.3 | 5.2 | 3.2 | 2.0 | 0 |
| T.P., 2 | N | 30 | 22.3 | 15.0 | 27.0 | 6.1 | 7.2 | 3.0 | 4.3 | 4.4 | 2.0 | 4.4 | 2.7 | 1.5 | 0.2 |
| T.P., 3 | H | 44 | 52.0 | 14.3 | 28.0 | 5.4 | 8.9 | 3.3 | 4.6 | 4.4 | 1.4 | 3.8 | 2.3 | 1.3 | 0.2 |
| T.P., 4 | I | 49 | 80.9 | 9.3 | 30.3 | 5.2 | 10.3 | 3.9 | 4.8 | 4.3 | 1.8 | 3.5 | 2.3 | 1.1 | 0.1 |
| J. J., 1 | N | 57 | 87.9 | 7.7 | 31.8 | 6.1 | 13.1 | 5.2 | 2.1 | 4.2 | 1.1 | 2.3 | 2.0 | 0.2 | 0.1 |
| J.J., 2 | H | 70 | 79.5 | 7.9 | 34.0 | 7.4 | 12.4 | 6.5 | 2.3 | 4.2 | 1.2 | 2.1 | 1.5 | 0.1 | 0.5 |
| J.J., 3 | I | 90 | 89.2 | 6.4 | 37.9 | 7.6 | 14.7 | 7.3 | 2.0 | 4.9 | 1.4 | 1.9 | 1.4 | 0 | 0.5 |
| J.D.M., 1 | N | 3 | 78.7 | 13.9 | 26.7 | 4.8 | 10.6 | 4.0 | 3.6 | 2.8 | 0.9 | 4.5 | 3.9 | 0.5 | 0.1 |
| J.D.M., 2 | H | 41 | 82.3 | 12.9 | 28.8 | 4.7 | 12.2 | 3.9 | 3.3 | 3.9 | 0.8 | 3.0 | 2.1 | 0.8 | 0.1 |
| J.D.M., 3 | I | 89 | 91.8 | 7.2 | 32.6 | 6.7 | 14.2 | 6.5 | 1.9 | 2.6 | 0.7 | 1.5 | 1.1 | 0.1 | 0.3 |
| J.M., 1 | N | 21 | 72.0 | 16.8 | 25.9 | 3.9 | 9.7 | 3.7 | 4.4 | 3.1 | 1.1 | 5.9 | 3.8 | 1.8 | 0.3 |
| J.M., 2 | H | 77 | 85.3 | 11.8 | 34.1 | 6.1 | 14.2 | 6.2 | 2.3 | 4.3 | 1.0 | 4.4 | 3.1 | 0.5 | 0.8 |
| J.M., 3 | I | 109 | 110.2 | 8.2 | 37.5 | 7.2 | 15.4 | 6.4 | 1.9 | 5.1 | 1.5 | 2.4 | 1.5 | 0.4 | 0.5 |
| D.S., 1 | H | 46 | 68.2 | 16.1 | 27.7 | 3.5 | 10.6 | 3.5 | 6.3 | 3.1 | 0.7 | 4.7 | 1.8 | 2.7 | 0.2 |
| D.S., 2 | I | 53 | 69.5 | 16.8 | 25.3 | 3.5 | 10.7 | 3.2 | 4.6 | 2.5 | 0.8 | 4.5 | 2.6 | 1.7 | 0.2 |
| D.H., 1 | I | 81 | 119.8 | 6.4 | 31.3 | 3.5 | 15.0 | 6.1 | 2.0 | 3.7 | 1.0 | 1.9 | 1.3 | 0.4 | .02 |

* N = nonstimulated, H = histamine stimulation, I = insulin stimulation.

− 0.746). Next, the relationships between the original mEq/L of HCl and the chromatographic data were studied. There was a positive correlation between the mEq/L of HCl and the total area of the six protein-containing peaks ($r = + 0.874$), and with the individual peaks II ($r = + 0.838$) and III ($r = + 0.839$); there was an inverse correlation, however, with protein peak IV ($r = - 0.700$). As might be expected, there was a negative correlation between the mEq/L of HCl with the total hexoses eluted ($r = - 0.760$) and the hexose in H-I ($r = - 0.800$); however, there was a positive correlation of the mEq/L of acid with the hexose in H-V ($r = + 0.636$). All of the above r values have a $p < 0.01$ under the null hypothesis. There was no correlation between the acidity values and peak H-IV.

## DISCUSSION

The method developed by Richmond and coworkers (2) for the fractionation of pooled human gastric juice on the Amberlite IRC-50 resin column has been modified to achieve a more rapid and standardized separation. The composition of the protein-containing peaks has doubtless been changed to some extent by these modifications so that the chromatograms can not be strictly compared with those previously reported (2, 6). For example, in the earlier chromatograms blood group substance A was concentrated prior to the first protein peak (2) and it is now contained in peak I. Also, in the earlier chromatograms the protein material eluted from the column after the proteolytic enzymes resolved itself into only two peaks (2). By the present method it has been fractionated into three peaks. One of these, peak IV, appears to have some biological significance, since it varies inversely with the free acid of the original gastric juice. Finally, whereas hexose was formerly found in only one area (2), it is at present separated into three peaks. The method is now adaptable to chromatographing individual samples of human gastric juice and to comparing the resultant patterns.

The size of peaks II and III appears to give an index of the activity of the two proteolytic enzymes, pepsin and gastricsin, respectively, in comparing chromatograms. The protein and hexose content of the various peaks has a predictable relationship to the acid concentration in the original whole gastric juice. Glass (13), using paper electrophoresis, has demonstrated a similar relationship between his patterns and the gastric acidity. The notching of peak V observed in three of the chromatograms from normal individuals is being explored in an effort to gain further resolution, perhaps with the appearance of an additional peak.

Current studies are directed toward exploring the possible biological activity of the protein-containing peaks, not containing proteolytic enzymes, and to define the chromatographic patterns in various disease states.

## SUMMARY

The resin column technic has been modified to provide a more rapid method for fractionation of the nondialyzable components of human gastric juice, adaptable to individual specimens. The method provides, furthermore, a more complete separation of the peaks from one another so that intraindividual and interindividual variations are comparable.

Six distinct and fairly symmetrical protein-containing peaks were identified in chromatograms from normal individuals. Hexoses were eluted in association with protein peaks I, IV, and V. Blood group substances A or B, when present, were eluted only in the area of peak I. Proteolytic activity was represented in peaks II and III only, corresponding to the two enzymes, pepsin and gastricsin. The size of the various protein and hexose peaks in the chromatogram of the dialyzed material correlated in a striking way with the concentration of HCl in the original gastric juice specimens. It is of special interest that the concentration of protein in peak IV correlated inversely with the concentration of acid in the original juice.

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