Serum Amylases, Isoenzymes, and Pancreatitis

I. Effect of Substrate Variation

Samuel Meites and Saul Rogols

Four plant starches were used to study human amylase activity in normal serum and urine, "pancreatitis" serum, duodenal fluid (secretin stimulated), saliva, and pancreatic extract. The starches were derived from waxy maize, high amylose corn, potato, and corn (pearl), and were lihtnerized. It was shown that the rate of digestion of starch by each of the fluids tested depends on the plant starch selected as substrate. Digestion of waxy maize was most rapid. The advantages of using waxy maize as a substrate are indicated as a means of markedly enhancing the sensitivity of serum amylase determination. It was also found that normal serum, urine, and saliva digested potato starch at a greater rate than corn starch with few exceptions, while pancreatitis serum, secretin-stimulated duodenal fluid, and pancreatic extract digested corn starch at a greater rate than potato. These findings suggest that organ-specific amylases exist, and that plant starches might be used to distinguish them.

Recent reports (1–5) on human amylase reaffirm older findings that "normal" serum amylase probably represents a mixture of enzymes which may be "organ-specific." Amylase in serum is attributed chiefly to the salivary glands and pancreas, and possibly to the liver. At least two "isoamylases" are found in normal human urine (6, 7). Salivary amylase is separable into several multimolecular forms (8, 9). Physical methods for determining organ-specific amylases in serum and urine are in the early stages of development. For this reason, we suggested (10, 11) that the natural variation in the chemical and physical properties of different plant starches used as substrates could be a means of distinguishing the amylases present in normal and perhaps in "ab-
normal” serum, the latter considered to be any serum showing elevated amylase activity.

This report presents evidence that (1) the rate of digestion by serum amylase depends on the plant starch selected as substrate, and (2) that the comparative rates of digestion of corn and potato starches by normal serum are reversed for serum obtained from patients with acute pancreatitis. Normal serum usually shows the pattern of digestion characteristic of saliva and normal urine, whereas the pancreatitis serum shows the pattern of digestion of duodenal fluid (secretin-stimulated), and of a saline extract of human pancreas.

Materials and Methods

Preparation of Starches

This study involved four commercial unmodified starches, as seen in Table 1: waxy maize, high amylose corn, potato, and corn (pearl). Each starch was linterized (acid modified) as follows (12): A 40% (w/v) suspension is prepared in water, titrated to 0.65 ± 0.10 N with concentrated hydrochloric acid, and stirred constantly for 12 hr. at 40 ± 2°. The suspension is then slowly brought to pH 6.8–7.0 with 1 N sodium hydroxide. If excess alkali is inadvertently added, it is best to discard the starch and prepare a new batch. Slow neutralization prevents “alkali-cooking,” which markedly alters the internal structure of starch. The starch suspension is then filtered with mild suction, using Whatman No. 1 paper. The starch is washed as necessary by resuspension in distilled water and is filtered until the filtrate is essentially free of reducing substances in the test procedure. Starch

Table 1. Properties of the Four Starches Used in This Study

<table>
<thead>
<tr>
<th>Starch</th>
<th>Amylose/amylpectin</th>
<th>Protein*</th>
<th>Lipids*</th>
<th>Ash*</th>
<th>Intrinsic viscosity (alkali fluidity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (pearl)†</td>
<td>40/60</td>
<td>0.422(0.407)</td>
<td>0.248(0.110)</td>
<td>0.199(0.090)</td>
<td>0.60(2.44)</td>
</tr>
<tr>
<td>Potato‡</td>
<td>30/70</td>
<td>0.145(0.087)</td>
<td>0.153(0.140)</td>
<td>0.260(0.260)</td>
<td>0.32(1.95)</td>
</tr>
<tr>
<td>Waxy maize§</td>
<td>15/85</td>
<td>0.421(0.082)</td>
<td>0.185(0.160)</td>
<td>0.090(0.222)</td>
<td>0.92(3.32)</td>
</tr>
<tr>
<td>High amylose corn</td>
<td></td>
<td>80/20</td>
<td>0.706(0.071)</td>
<td>0.180(0.170)</td>
<td>0.065(0.230)</td>
</tr>
</tbody>
</table>

Values in parentheses were obtained after acid-modification. Amylose/amylpectin values are those of the manufacturers. They presumably remain unchanged after acid-modification (12). All other values were obtained in the authors’ laboratory. The methods for protein and ash are in reference (13), the method for lipids in (16), and for intrinsic viscosity in (17).

* Values given as grams per 100 gm. dry weight.
† Corn Products Co., Argo, Ill.
§ American Maize Products Co., Roby, Ind.
|| National Starch and Chemical Co., Plainfield, N. J.
“blanks” (0.5 ml.) with absorbances as high as 0.2 in the test procedure have been used successfully. After drying at 20–24° for 12 hr., this starch should have a final moisture content of 12 ± 1% (13). Microscopic examination of starches prepared in this manner revealed intact granules with no apparent alteration of structure.

Preparation of Substrates

Each substrate was prepared with a slight modification of the method of Searcy et al. (14): One gram of lintnerized starch calculated on a dry weight basis is suspended in 100 ml. of phosphate-saline buffer pH 7.0, and placed in a boiling water bath for 2 hr.; it is then allowed to cool to room temperature. To preserve the suspension, it is prepared in a vaccine bottle plugged with a rubber diaphragm. The bottle of starch suspension is stored in a refrigerator (2–10°) for a maximum of 7 days and then discarded. After thorough mixing (necessary for all suspensions except waxy maize), starch suspension is withdrawn aseptically, as needed. The mild lintnerization treatment apparently does not prevent slow flocculation of all the starches used except waxy maize, despite the 2-hr. heating period.

Specimens for Analysis

Pancreatic extract was prepared by adding 500 ml. of 0.85% (w/v) saline to 35 gm. of pooled pancreatic tissue obtained at autopsy from 4 subjects, and grinding at high speed in a Waring blender for 10 min. The extract was twice passed through a nylon screen (100 mesh per inch) and then frozen until used.

Three duodenal fluid specimens were obtained from a subject undergoing a secretin test. These fluids contained between 50,000–60,000 Somogyi units of amylase activity per 100 ml.

Pancreatitis serum was collected from patients tentatively diagnosed as having acute pancreatitis. Six samples showing 800 or more Somogyi units of activity per 100 ml. were pooled and used for most of this study. Other pooled and individual samples were also used.

The “normal” serum, urine (random), and saliva specimens used to obtain the data for Table 2 were supplied by seemingly healthy young adult individuals. Other normal specimens were also used in the course of this study.

All specimens were stored frozen until used.

Procedure

The saccharogenic ultramicromethod of Searcy and coworkers (14, 15) was employed. Dilutions of specimens for analysis were selected on
Table 2. Digestion of Corn and Potato Starches by Normal Serum, Urine, and Saliva Specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Dilution, saline (v/c)</th>
<th>Net absorbance at 60-min. digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Corn starch</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>none</td>
<td>0.558</td>
</tr>
<tr>
<td>2</td>
<td>none</td>
<td>0.708</td>
</tr>
<tr>
<td>3</td>
<td>none</td>
<td>0.646</td>
</tr>
<tr>
<td>4</td>
<td>none</td>
<td>0.553</td>
</tr>
<tr>
<td>5</td>
<td>none</td>
<td>0.669</td>
</tr>
<tr>
<td>6*</td>
<td>none</td>
<td>0.552</td>
</tr>
<tr>
<td>7†</td>
<td>none</td>
<td>0.690</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1/3</td>
<td>0.470</td>
</tr>
<tr>
<td>2</td>
<td>1/4</td>
<td>0.449</td>
</tr>
<tr>
<td>3</td>
<td>1/4</td>
<td>0.573</td>
</tr>
<tr>
<td>4</td>
<td>1/5</td>
<td>0.580</td>
</tr>
<tr>
<td>5</td>
<td>none</td>
<td>0.608</td>
</tr>
<tr>
<td>6</td>
<td>none</td>
<td>0.480</td>
</tr>
<tr>
<td>7</td>
<td>1/1</td>
<td>0.638</td>
</tr>
<tr>
<td>8</td>
<td>1/1</td>
<td>0.476</td>
</tr>
<tr>
<td>Saliva (pool of 10 individuals)</td>
<td>1/1500</td>
<td>0.510</td>
</tr>
<tr>
<td></td>
<td>1/2000</td>
<td>0.264</td>
</tr>
</tbody>
</table>

* The reversal of digestive activity in this subject is exceptional; her urine (No. 8), however, did not show the reversal.
† This specimen was obtained from a healthy 6-months pregnant young adult woman. Note the reversal of digestive activity.

the basis of their activity-dilution curves as indicated in Fig. 2 (see Discussion). The dinitrosalicic acid reagent was prepared freshly whenever 1.0 ml. of the already prepared reagent heated in a boiling water bath for 5 min. and read against water as a blank, gave an absorbance value above 0.20. The reagent was generally stable for 7–10 days when stored in an amber bottle at room temperature. All absorbances were read at 540 mµ in a Gilford Model 300 Micro-Sample spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio.).

Results and Discussion

Acid-modification of starches makes them more suspendible in the substrate medium as well as more digestible by amylase. The luntnerization process used in this study is milder than that used commercially, judging from the increased speed of flocculation from suspension of corn, potato, and high amylose corn starches.

Since corn starch is commonly used in serum amylase determinations, we obtained data with it for Fig. 1, confirming the findings of Searcy and coworkers (14, 15) that at 1% (w/v) concentration, en-
zyme activity is independent of substrate concentration within certain limits (presumably up to 500 Somogyi units of activity per 100 ml.) For each fluid tested, a dilution curve was run in corn starch (Fig. 2). The dilution finally used was on the right limb of the linear curve, with absorbances falling between 0.3 and 0.4 at 60-min. digestion times. The dilution was assumed to be a valid one if the amylase activity was linear with time in corn starch (Fig. 3 and 4), indicating that zero-order kinetics may have prevailed. As it turned out, the activity was linear with time for each of the fluids studied (at the appropriate dilution) in all four substrates. The importance of making the proper dilution of the fluid to be tested cannot be overemphasized. Because the corn and potato starches used in this study were acid modified.

**Fig. 1 (top).** Variation of serum amylase activity with corn starch concentration in substrate. Values for net absorbance were obtained after 60-min. digestion. **Fig. 2 (bottom).** Typical activity-dilution curve for fluids containing high amylase concentrations. Dilution of each fluid finally used in experimental work was obtained from the right linear portion and had an absorbance between 0.3 and 0.4 at 540 mμ after 60-min. digestion.
differently from the commercial process, there are, at this moment, no established upper and lower limits of amylase activity for which the method may be deemed valid. Use of that dilution giving an absorbance value between 0.3 and 0.4 was arbitrarily selected, and
differentiated from the commercial process, there are, at this moment, no established upper and lower limits of amylase activity for which the method may be deemed valid. Use of that dilution giving an absorbance value between 0.3 and 0.4 was arbitrarily selected, and
gave consistent values when activities in the four starches were compared. Only in the case of the normal serum and urine specimens (Table 2) were some absorbances greater than 0.4 after 60 min. of digestion.

It should be pointed out that, ideally, comparison of enzyme activities in different fluids should be made with purified preparations rather than dilutions. Chromatographic and electrophoretic separation of amylases may soon make this approach possible. On the other hand, practical considerations dictate that we presently work with less than ideal conditions (18).

**Fig. 3 (top).** Typical variation of amylase activity with starch substrate. The linearity and the same order of digestive activity in the three substrates were obtained for all six fluids tested, at the proper dilutions. (Activity in potato starch is not included because of the variability indicated in Fig. 4). **Fig. 4 (bottom).** Variation in digestive rates of various fluids in corn and potato starch substrates. Group A is typical of pancreatic extract, duodenal fluid (secretin-stimulated), and pooled pancreatitis serum. Group B is typical of saliva, normal urine, and normal serum.
Our data reveal two important findings concerning human amylase: First, as Fig. 3 demonstrates, the rate of digestion of starch depends upon the plant source used; second, the comparative rates of digestion of corn and potato starches vary with the origin of the amylase.

There has been much conflicting evidence in the past as to whether the rate of digestive activity of serum amylase varies with the starch used. This subject was reviewed by Henry (19) and Henry and Chiamori (20). Their finding that there were essentially no differences between commercial starches was based on the use of a modified saccharogenic Somogyi technic (20, 21) which appears to be a considerably less sensitive method than Searcy’s for the detection of reducing substances. In addition, the starches used by Henry and Chiamori were not obtained as unmodified starches and then lintnerized in a uniform manner. This lack of uniformity in the preparation of starches could, in part, account for much of the conflicting data in the literature.

Waxy maize is, by far, the most rapidly digested of the four starches used in this study. It is saccharified at more than twice the rate of corn and potato starches. High amylase corn is digested at rates intermediate between waxy maize and corn and potato. It particularly should be noted that waxy maize, at a concentration as high as 1.5% (w/v), and possibly higher, forms a much more stable suspension than the other starches. In view of the digestive rate of amylase in waxy maize (Fig. 3), this genetically derived starch may serve to replace corn as a more sensitive as well as stable substrate for serum amylase determination. We are currently working on this possibility.

Until the relationship is clarified between organ-specific amylases and their existence as multimolecular forms within one organ, we have considered all human α-amylases to fall within the province of isoenzymes. While human α-amylases seem to have the same substrate specificity (qualitatively), the plant starches used are uniquely different because of their secondary chemical and physical properties accompanying a rather similar carbohydrate composition. For this reason, it is apparent that the rates of digestion may vary quantitatively with the amylase under study. The digestive rates of the fluids tested in this report are greater with waxy maize and high amylase corn than with potato and corn starches. This lends support to the idea that differential digestion obtained with Group A and Group B fluids is not due to carbohydrate content per se (see Table 1 for amylase/amylpectin composition), but is due to other factors. This challenging suggestion needs further exploration.

Figure 4 shows that amylase in biologic fluids can be classed into
two groups: Group A—secretin-stimulated duodenal fluid, pancreatic extract, and pancreatitis serum—digests corn starch at a greater rate than potato starch; Group B—saliva, normal urine, and serum—usually digests potato starch at a greater rate than corn starch. In a single study with the method of Henry and Chiamori (20), we observed the same reversal of activity by normal and pancreatitis serum in the two starches. These results suggest that salivary amylase is the predominant amylase in normal serum and urine, while in the other fluids pancreatic amylase is most prevalent. Alternative explanations of the reversal of activity, however, may be easily made—e.g., neither amylase may be predominant, but activity varies because of inhibitors, or differences in the physical and chemical properties of the two starches. Obviously, it would be desirable to test extracts of several additional tissues as well as sera possessing abnormal values, regardless of apparent cause. In general, serum amylase levels are not ordinarily believed to be elevated in people with liver disease, but this may reflect the choice of substrate used. This possibility must be explored. The fact that human serum amylase activity in corn and potato starches is variable, depending upon disease states, suggests that if both substrates could be used, greater specificity in amylase determinations might be achieved. Since other organ-specific amylases may be involved, however, further study of commercially available plant starches is apropos.

References


