THE LACTASE ACTIVITY OF THE INTESTINAL MUCOSA OF THE DOG AND SOME CHARACTERISTICS OF INTESTINAL LACTASE

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The early work on intestinal lactase has been reviewed by Plim-mer (1906) and Oppenheimer (1925). No uniformity of opinion was reached regarding the presence and distribution of this enzyme in the intestinal mucosa or in intestinal juice. Without attempting to review these investigations again, it suffices to state that some authors found lactase in the small intestine of young animals. Others reported its presence in the intestine of adult animals as well.

It is well known that animals do not utilize lactose readily when it is administered parenterally. When this sugar is given by mouth, however, it is absorbed and utilized. Unless the quantity fed is large, it is not excreted in the urine to any extent. Thus, all evidence points to the probability that lactose given per os is normally hydrolyzed before it is absorbed (Corley, 1927). Therefore, attempts to account for lactose absorption in terms of the observed lactase activity have not been successful. Röhman and Nagano (1903) in their extensive study of sugar absorption in dogs found no lactase activity in intestinal juice and only slight activity in the mucosa. In the Thiry loops of their dogs the observed lactase activity was totally inadequate for the hydrolysis of the lactose absorbed from the loops. They concluded that this sugar was not hydrolyzed prior to its absorption as were sucrose and maltose. Cajori (1933) found that lactose was absorbed from intestinal loops of dogs much more rapidly than could have been predicted from the enzyme activity of the intestinal juice.

The analytical methods available to the early investigators were
Intestinal Lactase

not satisfactory. Probably much of the uncertainty about intestinal lactase is ascribable to the analytical difficulties encountered in determining small quantities of glucose and galactose in the presence of larger amounts of lactose. The recent method of Tauber and Kleiner (1932-33) for the determination of monosaccharides has proved to be an adequate tool for quantitative lactase studies. Employing this method, we have reinvestigated the distribution of lactase in the adult dog's small intestine. The results which have been obtained are pertinent to discussions of the nutritive value of lactose (Koehler and Allen, 1934), for we have found lactase in quantities sufficient to account for the hydrolysis of considerable amounts of lactose in the small intestine.

Few studies have been published in which the characteristics of intestinal lactase have been investigated. Added information about this enzyme and the conditions influencing its action is presented in this paper.

EXPERIMENTAL

Enzyme Sources—Intestinal mucosa, stripped from the duodenum or jejunum of adult dogs, when employed directly without extraction, was prepared for use by grinding weighed samples of the fresh tissue with sand.

Water extracts were also used. They were prepared by extracting mucosa in the cold with an equal weight of toluene water for several days. After filtering through cotton or centrifuging, a turbid, milky extract was obtained which was used without further treatment, except in experiments involving adsorbing agents. These crude extracts kept well at 3° with only moderate decreases in enzyme activity during several weeks.

Intestinal juice was obtained from dogs with Thiry loops1 and was centrifuged before use.

Either water extracts of fresh dog liver were used or extracts of liver after dehydration and defatting with acetone and ether.

Enzyme Activity—The digestion mixtures usually consisted of 1 volume of 5 per cent (0.14 M) lactose solution, 1 volume of 0.2 M buffer solution, acetic acid-sodium acetate at pH 5.6, and 0.5 vol-

1 For the collection of this juice, Dr. Ravdin and Dr. Johnston of the Department of Research Surgery kindly permitted us to use their fistula dogs.
ume of enzyme solution or the weighed tissue. For monosaccharide analysis, 5 cc. portions were removed immediately after addition of the enzyme and at intervals afterwards. The mixtures, preserved with toluene, were kept at 38°, usually for digestion periods of 1 to 4 hours.

Enzyme solutions, inactivated by heat, were employed as controls in the usual way. No increase in monosaccharides was noted in these control experiments. In some trials with fresh tissue and with liver extracts, substitution of water for the lactose solution revealed an increase of monosaccharides, or substances oxidized by copper acetate. In these cases suitable correction was applied to the lactose results. The formation of reducing substances in intestinal extracts or juice was not observed.

Monosaccharide Analysis—In preparing a filtrate for the sugar determination, colloidal iron was found convenient. 1 cc. of colloidal iron (Merck, 5 per cent) and a drop of 20 per cent Na₂SO₄ were added to the 5 cc. sample removed from the digestion mixture. The precipitated ferric hydroxide was first separated by centrifuging and filtering and then repeatedly washed with water. The enzyme activity was removed, but not destroyed, by the ferric hydroxide and it was found important to avoid delays in separating the solution and the precipitate. The filtrate was made up to a volume of 50 or 100 cc. and 2 cc. aliquots were used for the monosaccharide determination, according to the directions of Tauber and Kleiner.

The electrolytes, acetates and phosphates, which were used as buffers in the digestion mixtures did not affect the copper reagent in these dilutions. At greater concentrations, phosphates interfered seriously with the Barfoed reagent.

With this method very little color was developed from the lactose present. For this reason it proved especially valuable when enzyme action was weak.

**Results**

**Distribution of Intestinal Lactase**—Typical results, obtained when lactose was digested with intestinal mucosal tissue and succus entericus, are given in Table I. It will be noted that the maximum lactase activity was obtained when fresh, finely ground tissue was used. Jejunal mucosa revealed a 10 to 30 per cent greater lactase
activity than duodenal tissue. In the four experiments in which duodenal and jejunal mucosa are compared, the two tissues, after removal from the animal, were subjected to identical treatment before and during the digestion trials. Water extracts of mucosa uniformly showed less enzyme activity than the tissue. Feeble enzyme activity was found in jejunal juice, the range of activity of six juices tested being 0.5 to 3.1 mg. of lactose hydrolyzed per cc. of juice per hour. Juice from a Thiry loop of the colon failed to induce lactose cleavage.

The observation that water extracts of mucosa are less active than the unextracted tissue and that the succus entericus exhibits only slight lactase activity suggests that this enzyme is intimately associated with mucosal cells. This conclusion that the succus entericus is a digestive fluid of only minor importance was recently reaffirmed for a number of enzymes of the small intestine by Cajori (1933). The major digestive action, resulting from enzymes elaborated in the small intestine, would then occur intracellularly or in direct contact with the mucosa.
The lactase activity here reported is less than the maltase and sucrase activity of the intestinal tissue of dogs found by Röhman and Nagano (1903). It is important to determine whether the observed lactase is sufficient to account for the hydrolysis of lactose when this sugar is absorbed from the gut. Cajori (1933) observed in the dog an average absorption of 435 mg. of lactose in 1 hour from a 22 cm. Thiry loop of the jejunum. The area of this loop was estimated to be 88 sq.cm. and to contain 12.5 gm. of mucosa. This latter estimate was based on an average yield of 0.14 gm. of mucosa per sq.cm. of gut. If the 435 gm. of absorbed lactose were hydrolyzed, each gm. of mucosa hydrolyzed 35 mg. of lactose. This is probably a maximal value, for it is quite likely that under the extreme conditions of this experiment some lactose disappeared from the loop unhydrolyzed. The loop is flooded with sugar solution, a condition which must rarely occur during ordinary feeding. It will be observed in Table I, nevertheless, that an occasional active tissue preparation exhibited a lactase activity of this order. This seems significant, for the actual lactase activity of tissue functioning in situ is undoubtedly greater than can be demonstrated experimentally. Conditions during testing of enzyme activity must only approximate those existing during digestion and absorption.

In view of these findings one is not forced to conclude, as Röhman and Nagano did, that lactose is necessarily absorbed without being hydrolyzed. However, in this connection it is of some interest to record the finding of slight lactase activity in liver tissue and in water extracts of liver. Although liver rarely hydrolyzed more than 1 mg. of lactose per hour per gm. of tissue, the finding of a liver lactase may be of significance in revealing a further mechanism for lactose hydrolysis in the body.

Characteristics of Intestinal Lactase—Water extracts of intestinal mucosa were used in studying the characteristics of lactase and the factors influencing its action.

pH Optimum—In determining the pH optimum, 0.2 M acetate or phosphate buffers were used. In the critical range, the pH of the buffers and certain of the digestion mixtures was determined with the glass electrode.2

2 We are indebted to Dr. E. J. de Beer for the measurements with the glass electrode.
Dog intestinal lactase exhibited optimum activity (Chart 1) in solutions of pH 5.4 to 6.0. This optimum pH is similar to that found for intestinal lactase in other animals, but is distinctly different from plant lactases. Freudenberg and Hoffman (1922) state that calf intestinal lactase had an optimum activity at about pH 5.0. Wigglesworth (1927) reported a pH optimum range of 5.0 to 6.4 for gut lactase of the cockroach. Willstätter and Oppenheimer (1922) found that yeast lactase had a pH optimum at 7.0, whereas pH 4.2 was optimum for almond lactase according to Willstätter and Csányi (1921).

Reaction Course—Armstrong (1904) studying the hydrolysis of lactose with lactase from kephir-grains observed that the monomolecular reaction constant increased at first, and decreased later. The same thing was found in the case of yeast lactase by Willstätter and Oppenheimer (1922). In our experiments with intestinal lactase, we followed the hydrolysis of lactose at intervals during a 24 hour period. It is interesting to note (Table II) that a similar monomolecular reaction course was observed.

Effect of Substrate Concentration—The initial velocity of lactose hydrolysis was found to decrease when the lactose concentration
in the digestion mixtures was less than 2 per cent (0.056 M). At a lactose concentration of 0.006 M the initial velocity was about one-half of the maximum observed with higher lactose concentrations.

Analysis of the data by graphical methods indicated that this value, 0.006 M, may be regarded as a true Michaelis constant and that the mechanism of lactase action involves the combination of 1 molecule of lactose and 1 molecule of enzyme. For evaluation of the dissociation constant, $K_s$, in the simplest case of enzyme-substrate combination, $S + E \rightleftharpoons SE$, Lineweaver and Burk (1934)

### Table II

**Lactose Hydrolysis by Intestinal Lactase**

<table>
<thead>
<tr>
<th>t (hrs.)</th>
<th>Monosaccharides as glucose (mg.)</th>
<th>$x$ (lactose hydrolyzed) per cent</th>
<th>$K^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>30</td>
<td>4.0</td>
<td>0.0177</td>
</tr>
<tr>
<td>2.0</td>
<td>62</td>
<td>8.3</td>
<td>0.0188</td>
</tr>
<tr>
<td>3.0</td>
<td>92</td>
<td>12.3</td>
<td>0.0190</td>
</tr>
<tr>
<td>5.0</td>
<td>146</td>
<td>19.5</td>
<td>0.0188</td>
</tr>
<tr>
<td>7.0</td>
<td>191</td>
<td>25.5</td>
<td>0.0183</td>
</tr>
<tr>
<td>23.5</td>
<td>409</td>
<td>62.5</td>
<td>0.0181</td>
</tr>
</tbody>
</table>

* $K = (1/t) \log (100/(100 - x))$.

suggest the use of the linear form of the Michaelis-Menten equation:

$$
1 = \frac{K_s}{V_{\text{max}}} S + \frac{I}{V_{\text{max}}}.
$$

When the reciprocals of the velocities ($v$) were plotted against the reciprocals of the lactose concentrations ($S$) a straight line resulted. Following Lineweaver and Burk's method, the slope of the line $K_s/V_{\text{max}}$ was determined and $V_{\text{max}}$ obtained by straight line extrapolation. In Experiment 1, $K_s$ was calculated as 0.0055 and in Experiment 2 as 0.0061. The initial velocities at different lactose concentrations and the initial velocities calculated from the $K_s$ values 0.0055 and 0.0061 are given in Table III. The agreement
between the observed and calculated velocities justifies the extension of the Michaelis concept to this enzyme. This value for the Michaelis constant is very much lower than the $K_s$ for gut invertase (Cajori, 1939).

In the presence of glucose, lactase action was retarded, the decrease in lactose hydrolyzed in 22 hours amounting to 40 per cent. On the other hand, galactose in a similar period had practically no effect on lactase activity. In both experiments the initial molar concentration of monosaccharides was twice that of lactose. This result confirms the earlier observations of Stevenson (1912) who characterized gut lactase as glucolactase.

Freudenberg and Hoffman (1922) reported that phosphates accelerated the action of calf intestinal lactase. With dog mucosal extracts, we have been unable to observe that the addition of phosphate had any effect on the rate of lactose hydrolysis. At the same pH, identical amounts of lactose were hydrolyzed with acetate alone and with the acetate plus phosphate.

### Table III

**Initial Velocity of Lactose Hydrolysis at Different Lactose Concentrations**

<table>
<thead>
<tr>
<th>Lactose concentration</th>
<th>Lactose hydrolyzed in 4 hrs.</th>
<th>Relative initial velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.110</td>
<td>24.3</td>
<td>100</td>
</tr>
<tr>
<td>0.056</td>
<td>24.4</td>
<td>100</td>
</tr>
<tr>
<td>0.028</td>
<td>22.5</td>
<td>92</td>
</tr>
<tr>
<td>0.014</td>
<td>17.5</td>
<td>72</td>
</tr>
<tr>
<td>0.009</td>
<td>15.0</td>
<td>62</td>
</tr>
<tr>
<td>0.007</td>
<td>14.4</td>
<td>59</td>
</tr>
<tr>
<td>0.0035</td>
<td>9.3</td>
<td>38</td>
</tr>
<tr>
<td>0.002</td>
<td>6.0</td>
<td>25</td>
</tr>
</tbody>
</table>

| Experiment 2          |                             |                          |
| 0.056                 | 30.5                        | 100                      |
| 0.014                 | 23.1                        | 76                       |
| 0.006                 | 15.0                        | 49                       |
| 0.0044                | 11.9                        | 39                       |
| 0.0017                | 5.0*                        | 16                       |

$K_s = 0.0055$  
$K_s = 0.0061$

* Not used in the calculation of $K_s$. 

Relative initial velocity

- Calculated
- Observed

**Table**

- Initial velocity
  - Calculated
  - Observed

*Not used in the calculation of $K_s$.**
Adsorption—A few experiments are presented recording the behavior of intestinal lactase in the presence of adsorbing agents. When shaken for a few minutes with kaolin, extracts of intestinal mucosa, acidified with acetic acid, were considerably clarified. There was no loss of lactase activity. In alkaline solutions, kaolin removed considerable amounts of the enzyme. Treatment with kaolin served as a convenient method for partial purification of the crude mucosa extracts. The extracts, containing acetic acid, retained their activity although lactase was found to be very sensitive to strong acid. An extract made acid to Congo red with HCl was completely inactivated in a few minutes.

Ferric hydroxide or alumina cream completely adsorbed lactase from crude or clarified extracts, acidified with acetic acid.

Elution of the enzyme from these adsorbing agents was attempted with dilute NaOH, NH₄OH, or Na₂HPO₄ solutions. Elution was incomplete or accomplished with great loss of activity. Maximum recoveries of 20 to 25 per cent of the original lactase activity were obtained with 0.2 M Na₂HPO₄.

Some peptization of ferric hydroxide occurred during elution from this adsorbent and yellow solutions resulted. This was observed with freshly precipitated Fe(OH)₃ and with preparations that had been aged for a year.

The enzyme solutions obtained after the use of kaolin, adsorption, and elution gave positive biuret tests and contained heat-coagulable protein.

SUMMARY

A lactose-splitting enzyme has been found in the duodenal and jejunal mucosa of the dog, in water extracts of the mucosa and, to a lesser extent, in jejunal juice and in liver. The question of lactose hydrolysis in the intestinal mucosa in relation to lactose absorption has been discussed.

Intestinal lactase exhibited maximum activity in acid solution, between pH 5.4 and 6.0. Its activity was inhibited by glucose but not by galactose. The presence of phosphates had no effect on the lactase activity. A determination of the Michaelis constant revealed a $K_\text{m}$ value of 0.006.

Lactase was found to be adsorbed readily, from slightly acid solution, by aluminum hydroxide or ferric hydroxide.
BIBLIOGRAPHY

Röhm, F., and Nagano, J., Arch. ges. Physiol., 95, 533 (1903).
Stevenson, M., Biochem. J., 6, 250 (1912).
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