



## RECOVERY OF LACTASE ENZYME FROM CALF INTESTINE

by

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### ABSTRACT

The lactase from calf intestine was studied in 5 % lactose solution, inoculating 4.0 ml or 4.9 ml aliquot portions of this diluted sugar, tempered at 37° C in water bath, with 1.0 ml or 0.1 ml enzyme extract. The reaction monitored by analysing the glucose formed from hydrolysis of lactose by lactase present was tested, at different incubation times after inoculation with enzyme, using a YSI Model 27 Enzyme glucose Analyser.

Because no recent investigations about the variations in the lactase, along the whole calf small intestine, has been done, the determination of its content, in each 10 cm intestinal fragment from stomach, was carried out.

In order to estimate the best conditions, in terms of pH for separating and purifying lactase from crude liquors, using an insoluble matrix of DEAE-Cellulose in small batches, an adsorption procedure was evaluated.

## INTRODUCTION

Recovery and utilization of waste products from the agricultural and food processing industries in an economic and ecologically acceptable manner are becoming increasingly important.

Any waste material that is utilized must compete economically with products available or create their own markets. In some food industries the very existence of the plant may be dependent on the economic and ecological disposal of waste material (1).

The problems, for example, related to the disposal and utilization of cheese whey have received much national and regional attention. Legislation enacted in recent years for environmental protection prohibit dumping of cheese whey into nearly streams and lakes. These factors alone have forced many smaller cheese plants to close.

The most urgent need and most far-reaching benefits of the research in this field would be the massive reduction of pollution now caused by the dumping of whey into streams or into disposal systems ill equipped to handle it.

The alternative to disposal is utilization. Indeed, whey has long been recognized as a source of valuable nutrients.

The complete or near complete utilization of both sweet and acid whey would greatly enhance the world's food supply and help to answer the questions of survival in many underdeveloped and emerging countries.

By a membrane process, it is possible to separate the protein in whey from the milk sugar, lactose. The milk protein so produced has a ready market. What makes the overall process economically difficult to justify is the lack of a good use for lactose.

Crystalline lactose can be easily produced, but its potential market is quite limited. Lactose can also be fermented by a

class of bacteria to produce lactic acid which also have economic value.

Alternatively, lactose can be converted into the easily fermentable sugars, dextrose and galactose, by the enzyme lactase.

After treatment with an immobilized lactase/glucose isomerase system the sweetened whey wastes can perhaps be recycled as a food supplement (2).

The addition of lactase to milk so that the milk can be utilized by lactase deficient people is, also, a nutritional problem that needs to be solved (18).

The enzymes that bring about hydrolysis of the galactosidic linkage are widely distributed in both plant and animal tissues. They occur in the emulsions and tips of some Rosaceae members, in kefir grains, almonds, and seeds of soybeans, alfalfa and coffee (8, 9, 10).

The enzyme has been found to be present in the fungus *Aspergillus orizae*, (11) *Aspergillus niger*, *Aspergillus flavus* (12, 13) and *Neurospora sp.* (14).

Lactase has likewise been reported to be a normal constituent of the adult intestinal secretion in human.

A detailed review of the literature about the presence of lactase in the intestines of animals is given by Plymmer (7). The mucous membranes of calf have been studied and the results seem to indicate that lactase is present in the mucous membranes of the small intestine of young animals but disappears with increasing age. Fisher and Niebel (15) assumed that the lactase production in old animals might be provoked or increased by feeding for a longer period with milk.

The question of the distribution of lactase in different parts of the small intestines has been made the subject of investigations (16, 17). However, neither of these authors have proven significant variations in the lactase content along the small intestine. In addition no recent investigation into this matter has been done. Therefore, it is of interest to know if the small intestine of the calf is a real potential source of commercial

lactase ; its variation along the whole intestine, and the best conditions for separation and purification from crude liquors, using and insoluble matrix of DEAE-Cellulose is another point.

The recovery of the lactase from calf intestine and the problem aspects to be solved in such an extraction process were the main goals of this study.

## EXPERIMENTAL SECTION

*Materials.* Lactose-milk was obtained from J. T. Baker Chemical Co., (Phillipsburg, New Jersey). Calf intestines was generously provided by Johnston Dressed Beef and Veal Co., Inc. (Johnston R. I.). DEAE-Cellulose was obtained from Viscose Group Ltd., Development Division, (Foreshore, Swansea). Reagent grade chemicals and distilled water used throughout.

## METHODS

*Assay for Lactase Activity.* Lactase activity was assayed using a glucose analyser (YSI Model 27 Glucose Analyser — Yellow Springs Instrument Company, Inc. Yellow Springs, Ohio) and the definition of an activity unit at 37° C is identical to the one previously described by Dalhquist (19).

Each test was performed in 4.0 or 4.9 ml of 5% lactose solution in 0.1 N phosphate buffer at pH 6.5, contained in centrifuge tubes previously incubated at 37° C in a water bath. To these volumes of tempered lactose solution were added 1.0 or 0.1 ml of calf intestine extract containing lactase. The lactase activity was then followed by the glucose released from hydrolysis of lactose. The amounts of this monosaccharide were

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determined specifically by a YSI Model 27 Glucose Analyser unit. Twenty-five microliter aliquots of the reactivated mixture were tested for glucose at 0, 5, 10, 15, 30, 45 and 60 min. and then at hourly intervals until the values started to decline. The zero time test corresponded to the moment at which the substrate was inoculated with enzyme solution.

One unit of lactase is defined as the amount of enzyme that hydrolyses one micro-mole of lactose per minute at a temperature of 37° C at pH 6.5.

*Preparation of Calf Intestine Extract.* Calf intestines were received frozen from Johnston Dressed Beef and Veal Co., Inc. (Johnston, R. I.) and maintained frozen until be used. Intestines were thawed and then emptied and cleaned by rinsing for a short time in cold water and divested of fatty tissue. Then the mucosal lining of whole or of 10 cm fragments of washed calf intestine were mixed with equal weights of 0.1 M sodium phosphate buffer at pH 6.5, and immediately blended in a Waring Blender until a homogenous slurry was obtained. After blending, the homogenates were centrifuged at 10,000 r.p.m. at 4° C for 30 min. in a Automatic Refrigerated Centrifuge Sorvall Superspeed RC 2-B (Norwalk, Connecticut). Supernatants were stored in beakers at -20° C until removal for testing.

*DEAE-Cellulose Binding Study.* The two major procedures for separating and purifying enzymes from crude liquors are precipitation and adsorption. In this investigation, however, the adsorption procedure was used in order to determine its usefulness, and to study the best conditions for the adsorption isolation of the calf intestinal lactase in terms of pH and salt concentration, with an insoluble matrix of DEAE-Cellulose resin. Aliquots of DEAE-Cellulose were reactivated with 0.3 M NaOH and equilibrated with 0.02 M phosphate buffer to pH 6.5. This material was drained, and 1.0 g portions were weighed into two 100 ml beakers, one containing 10 ml of the enzyme extract diluted in 100 ml of distilled water, and other containing 10 ml

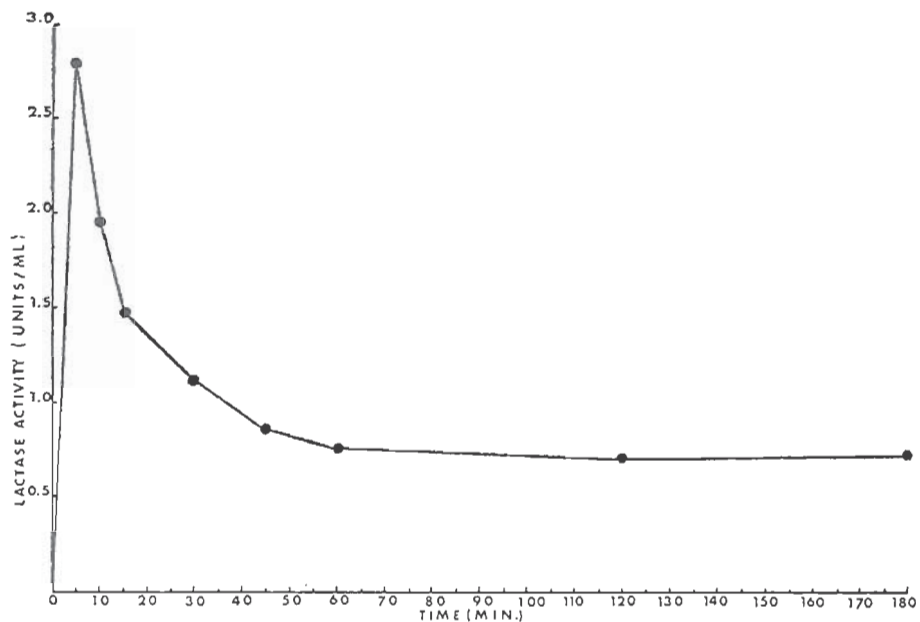


FIGURE 1—Effect of the inoculation time on calf intestinal lactase activity at pH 6.5 (Incubation temperature 37° C).

of the enzyme extract diluted in 500 ml of distilled water. The same procedure was followed at pH 6.0 and 6.5. In all batches, the resin was stirred with the diluted enzyme preparations for 1 hr and then allowed to settle until a clear supernatant resulted.

The activity of each enzyme supernatant was tested in duplicate before and after the binding treatments.

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RESULTS

*Effect of Reaction Time.* As expected, time influenced activity, as is shown in Figure 1. During the first five minutes, from the time of the inoculation, the activity increased from 0 to 2.78 units/ml, declining after that to values of 1.94, 1.48, 1.11, 0.86 and 0.78 units/ml at 10, 15, 30, 45 and 60 min., respectively (Table I). Then the activity was maintained at least for the next two hours, decreasing again after this incubation time. Figure 2 illustrates that the glucose produced by hydrolysis of

TABLE I  
LACTASE ACTIVITY

| <i>Inoculation Time (min.)</i> | <i>Lactase Activity (Units/ml)</i> |
|--------------------------------|------------------------------------|
| 0                              | 0                                  |
| 5                              | 2.78                               |
| 10                             | 1.94                               |
| 15                             | 1.48                               |
| 30                             | 1.11                               |
| 45                             | 0.86                               |
| 60                             | 0.78                               |
| 120                            | 0.69                               |
| 180                            | 0.69                               |
| 330                            | 0.64                               |
| 390                            | 0.63                               |
| 450                            | 0.60                               |
| 1.680                          | 0.56                               |
| 3.060                          | 0.47                               |

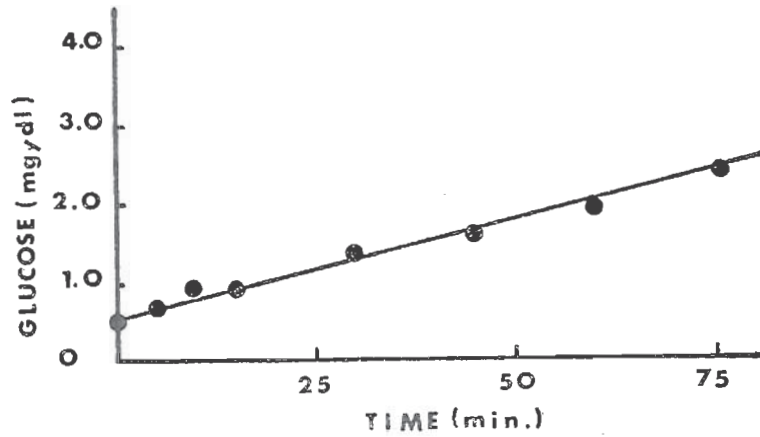


FIGURE 2—Effect of the inoculation time on lactase activity from calf intestinal, at pH 6.5 (Inoculation temperature at 37° C).

lactose increased gradually from 0.5 to about 2.5 mg/dl during an incubation time of 75 min.

*Distribution of Lactase along the Calf Intestine.* The enzyme activity as determined by the Glucostat Method, was plotted against distance of intestine from the stomach, as is shown in Figures 3 and 4. Lactase activity varied from 0.2 units/ml in upper third 10 cm portions of small intestine to 1.1 units/ml in the lower one third near the large intestine.

*DEAE-Cellulose Binding Study.* The data recorded in Table II shows that lactase can be recovered from aqueous solutions in which it is contained in a mixture with smaller organic molecules, by passing the solution through an ion-exchange resin, then eluting the resin with buffers at appropriate molarity, in order to obtain an enzyme rich fraction.

On the two buffers studied (pH 6.0 and pH 6.5) the first one offered the best binding conditions.

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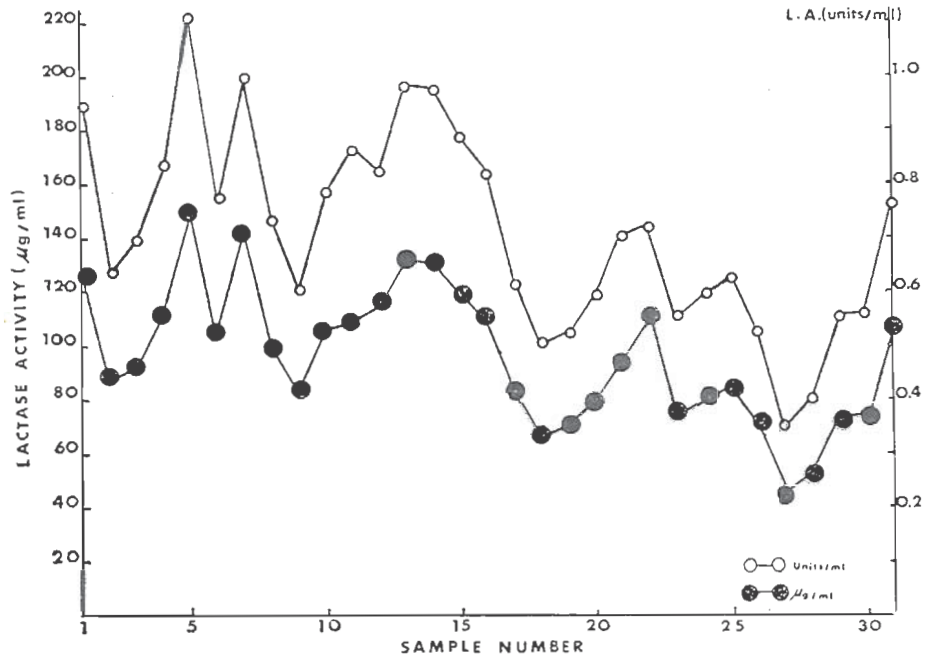


FIGURE 3 — Lactase activity of homogenates (10 cm mucosa-calf intestine) from the end of ileum to the 30th fragment by Glucostat method.

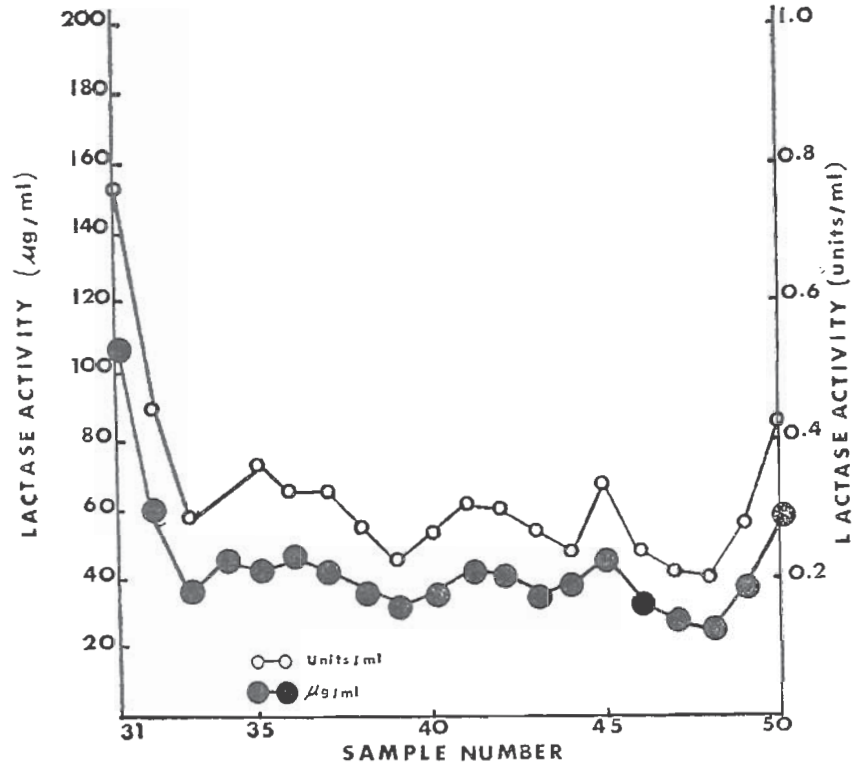


FIGURE 4—Lactase activity (Glucostat method) of homogenates (10 cm mucosa-calf intestine) from the 31st fragment to the duodenum.

### DISCUSSION

Lactase, the enzyme that breaks down lactose ingested in milk or a milk product, is a specific intestinal betagalactosidase that acts only on lactose, primarily in the jejunum, the second of the small intestines three main segments. It appears in

the differentiated cells, specifically within the brush border of the cells at the surface of the villus.

Lactase is not present in the intestine middle of the last stage of gestation (7). Its activity attains a maximum immediately after birth. Thereafter it decreases, reaching a low level, for example, immediately after weaning (4). It is extremely active after birth in intestine from calf, and then this activity decreases over the next few weeks.

Heilstov (4), showed that calf intestine contains two enzyme activities, although only one of which hydrolysis lactose (2, 4).

Adsorption techniques have achieved great importance in laboratory practice for isolating and purifying lactase. Ion-exchange materials, including commercially available anionic resins, seemed to be useful for that purpose.

Diethylamine Ethyl Cellulose (DEAE-Cellulose) was assayed in binding studies, and it is quite simple to set up a batch that selectively binds the enzymes from a solution flowing through the resin. The pH and salt concentration of the solution influenced the binding process.

According to these results it seems promising and quite simple to set up a column that selectively binds lactase from a solution flowing through the column and then eluting the enzyme progressively from the column by a program of buffers at different salt concentrations, pHs, or other gradients. By use of readily available automatic fractionation collectors the separated enzyme can be appropriately collected and subjected to further purification steps, if desired.

It has become a common practice in the laboratory, where efficient equipment is available to freeze dry quantities from a few milliliters up to several liters of liquid enzyme. This method can be used where enzyme inactivation is encountered in the precipitation and drying steps. It is, however, common practice in laboratory and in industry to store enzyme solutions under refrigeration, and then with little effort to later reduce them to dry powders.

## CONCLUSIONS

Activity of calf intestine lactase buffered at pH 6.5 increased from zero to five minutes inoculation time in lactose solution tempered at 37° C. The activity then declined rapidly every five minutes until 60 minutes after inoculation. The activity observed during this period (0-5 min.) varied from 0 to 2.78 units/ml and then declined from 2.78 to 0.75 units/ml (5 minutes to 60 minutes). Then the activity stayed at same level (0.75 units/ml) at least, for the next two hours.

Along the calf intestine the lactase was less active in portions near to stomach than in fragments distant from it; increasing from 0.2 units/ml to 1.1. units/ml.

The enzyme seemed to be more easily bound to DEAE-Cellulose resin buffered at pH 6.0 than at pH 6.5 (Table II).

TABLE II  
CALF INTESTINAL LACTASE  
(BINDING IN DEAE-CELLULOSE RESINE)

| <i>Binding Conditions</i> | <i>Enzyme Activity (Units)</i> |             | <i>Lactase bound</i> |
|---------------------------|--------------------------------|-------------|----------------------|
|                           | Original Extract               | Supernatant | %                    |
| 6.0                       | 21.66                          | 10.68       | 50.69                |
| 6.5                       | 15.10                          | 14.20       | 5.96                 |

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