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I am submitting herewith a thesis written by Foster Glen Hembry entitled "Glucose Absorption, Carbohydrase Activities, and Carbohydrate Hydrolysis and Uptake in the Intestinal Tract of Mature Sheep." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

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GLUCOSE ABSORPTION, CARBOHYDRASE ACTIVITIES, AND
CARBOHYDRATE HYDROLYSIS AND UPTAKE IN THE
INTESTINAL TRACT OF MATURE SHEEP

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CHAPTER I

INTRODUCTION

Carbohydrates are the predominant source of energy used to carry out the biological processes of growth, maintenance, and reproduction in the animal body. Glucose is the most important carbohydrate metabolite in both the nonruminant and the ruminant animal. In the nonruminant, glucose is also the most abundant end product of carbohydrate digestion.

The nonruminant is able to utilize only traces of cellulose and hemicellulose as end products of microbial degradation in the large intestine, and the small intestine of this animal is the major site of carbohydrate assimilation. The microbiological digestion of carbohydrates makes the reticulo-rumen and to a lesser extent the omasum the major sites of carbohydrate assimilation in the ruminant animal. These microorganisms also degrade a portion of cellulose and hemicellulose to absorbable end products which can be utilized by the host animal.

The rumen, being the major site of carbohydrate assimilation, has received the greatest attention by investigators of protein, fat, and carbohydrate utilization in the ruminant. Some research has been conducted in an attempt to determine the ability of the preweaned ruminant to utilize certain carbohydrates. Little information is available in the literature regarding the digestion of carbohydrates and the absorption of glucose in the small and large intestine of the mature ruminant animal.

In advanced pregnancy and during lactation an animal is in a critical glucose balance resulting from lactose synthesis (Armstrong, 1965). Since the major precursor of lactose is blood glucose (Reiss and Barry, 1953) supplied primarily through gluconeogenesis of noncarbohydrate sources (Armstrong, 1965), the importance of any glucose absorbed from the gastro-intestinal tract which would contribute directly to the maintenance of the blood glucose level is obvious.

Although it is estimated that only about 10 per cent of the glucose requirement of the ruminant animal is absorbed from the alimentary tract (Armstrong, 1965), it is of interest to know the efficiency of glucose absorption and utilization of other carbohydrates in the small and large intestine of the mature ruminant.

The objectives of these studies were to determine: (1) the levels of the carbohydrases, maltase, lactase, sucrase, amylase, and cellobiase in the mucosa of the duodenum, jejunum, and ileum; (2) the rate of absorption of glucose in vivo in the duodenum, jejunum, ileum, cecum, and colon; and (3) the degree of utilization of maltose, starch, and cellulose in the duodenum, jejunum, ileum, cecum, and colon in mature sheep.

CHAPTER II

LITERATURE REVIEW

I. CARBOHYDRATES IN THE SMALL INTESTINE

It is well established that microbial activity in the rumen constitutes the major digestion of carbohydrates in the mature ruminant and that digestion of carbohydrates in the small intestine is of secondary importance. Kameoka and Morimoto (1959) demonstrated the disappearance of 65 to 96 per cent of the digestible nitrogen-free extract from the rumen of goats. The nitrogen-free extract was converted to volatile fatty acids and absorbed, but they suggested that the remaining 4 to 35 per cent was possibly absorbed from the small intestine as glucose. Since conversion of carbohydrates to carbon dioxide and acetate is one of the primary pathways of energy metabolism in rumen microorganisms, this pathway along with other degradation pathways could lead to bacterial metabolic losses which would also result in something less than 100 per cent absorption of degraded carbohydrates.

There is evidence that free sugars and polysaccharides do enter the abomasum when an animal is maintained on a normal diet. Barhydt and Dye (1957) reported that the abomasal contents of calves on a 5:1 hay to grain ration contained 2 mg. of free sugar per gm. of dry matter, primarily consisting of pentoses, and that the hydrolyzable polysaccharides averaged 108 mg. per gm. dry matter of which 60 per cent were

pentose hemicelluloses and 40 per cent were hexose polysaccharides. The fermentable sugars were identified as glucose, fructose, and mannose. These investigators found 25 to 40 mg. of abomasal polysaccharides per gm. of dry matter in a 10-month-old calf fed an all alfalfa hay ration and 100 to 125 mg. of polysaccharides per gm. of dry matter in the same calf fed an all grain diet.

Before the rumen is fully functional, some of the solid food material presumably passes directly into the abomasum (Hill, 1961). Hill also observed that undigestible residues of concentrates are excreted more rapidly than those of hay and suggested that some of the most recently ingested food might pass to the abomasum. Hill (1961) noted that during fermentation in the rumen bacterial polysaccharides are formed and polysaccharide granules accumulate in some protozoa. The importance of the rumen microorganisms depends not only on their fermentative processes in the rumen, but also on the food material derived from the digestion of the organisms in the lower parts of the digestive tract. Heald (1951) estimated that the amount of bacterial and protozoal synthesized polysaccharides reaching the abomasum of sheep was about 208 to 250 mg. per hour.

The small quantity of sugars derived from the food, and from microbial polysaccharides, or any starch which may escape fermentation make up the carbohydrates available for digestion in the small and large intestine (Phillipson, 1948). Since little if any digestion, fermentation, or absorption takes place in the abomasum (Barhydt and Dye, 1957), it is obvious that nearly all sugars and polysaccharides entering

the abomasum pass on into the small intestine. The total amount of carbohydrates that leaves the abomasum of the sheep on a given ration has not been determined.

The rate of passage of ingesta from the abomasum to the duodenum is an important factor in determining the total quantity of carbohydrate entering the small intestine. Barhydt and Dye (1957) estimated a flow rate of 380 ml. per hour for 10-month-old calves on a milk diet. Phillipson (1948) reported a flow rate of 300 to 450 ml. per hour from the abomasum in adult sheep. Ash (1961) demonstrated that the passage from the abomasum was quite varied and that feed intake, gastric secretion, and abomasal outflow are all interrelated.

Although all of the factors have not been determined, the amount of carbohydrate escaping fermentation in the rumen and entering the abomasum and subsequently the duodenum can be calculated from: (1) the free hexose content; (2) the potential hexose formers or polysaccharides; (3) the presence of certain pentoses; and (4) the rate of passage of material through the abomasum (Phillipson, 1948).

II. CARBOHYDRASES OF THE INTESTINAL TRACT

Although some authors still feel that enzymes responsible for hydrolysis of carbohydrates in the small intestine are secreted into the lumen by some unidentified cells, most researchers feel that these hydrolytic enzymes are located in the area of the brush border of the epithelial cell and not secreted into the intestinal lumen. The origin of the enzyme activity in the lumen of the intestine results from

desquamated epithelial cells (Leblond and Walker, 1956).

Dollar and Porter (1957) observed high lactase activity and very low maltase and amylase activity in extracts of intestinal mucosa of young calves. With increasing age the amylase and maltase activities increased while the lactase activity decreased and no sucrase activity was observed at any age. Walker (1959) observed a similar pattern in lambs as well as demonstrating that mucosa cells of pigs contained several times the maltase and sucrase activity found in lambs. The levels of lactase were similar in both species, but the total pancreatic amylase activity of the pig was much greater than that of the lamb.

Huber et al. (1958) confirmed the findings of Dollar and Porter (1957) and Walker (1959) that intestinal lactase decreased between birth and 8 weeks of age in calves, but found little increase in maltase activity over this time. This is in contrast with the work of Dollar and Porter (1957 and 1959) who obtained a threefold increase in maltase activity between 2 and 8 weeks of age. Mucosal extracts from human jejunum and ileum yielded considerable maltase followed in order by sucrase, lactase, and cellobiase activities (Dahlqvist, 1964).

Little information is available concerning the carbohydrases of the small intestine of the mature ruminant, but one fact that appears to be well established is that the nonruminant animal contains a much higher level of all intestinal carbohydrases than the ruminant.

III. CARBOHYDRATE UTILIZATION IN THE INTESTINAL TRACT

Carbohydrate digestion studies by Dollar and Porter (1957) demonstrated that calves up to 4 weeks of age utilize only glucose and lactose and at 9 weeks are able to utilize appreciable amounts of maltose, but no starch or dextrans. Huber et al. (1961) using an analysis of the increased level of blood-reducing sugars in jugular blood to measure carbohydrate utilization, demonstrated an increase of 130 mg. per 100 ml. of blood when glucose and lactose were ingested by 3-week-old calves. Maltose gave an increase of 30 mg. per 100 ml. and sucrose and starch gave no response. In older calves (Huber et al., 1961) the increase in blood reducing sugars due to lactose ingestion dropped from 130 mg. per 100 ml. at 22 days of age to about 10 mg. per 100 ml. at 600 days of age. Glucose gave the greatest response of all carbohydrates at all ages ranging from an increase of 130 mg. per 100 ml. at 22 days to about 40 mg. per 100 ml. at 600 days. The greatest response to maltose ingestion came at 50 days of age, an increase of 70 mg. per 100 ml. of blood and dropped off to an increase of 10 mg. per 100 ml. at 600 days of age. Response to starch ingestion was low at all ages, an increase of only 5 to 10 mg. per 100 ml. of blood. Huber et al. (1961) interpreted their results as responses resulting from events in the tract caudal to the omasum since animals ranging in age from 22 to 136 days were fed substrate rations by mouth while animals from 227 to 600 days of age were fed substrate rations directly into the reticulo-omasal orifice via rumen fistula.

Velu, Kendall, and Gardner (1960) administered sugars orally to calves ranging in age from 1 to 14 weeks. Marked increases in blood reducing sugars resulted from administration of glucose, lactose, and galactose. However, the calves were unable to utilize sucrose and the utilization of maltose was comparatively low.

By employing a re-entrant fistula just cranial to the ileo-cecal junction and by administering separate oral diets containing sucrose and lactose to calves 1 to 4.5 months of age, Morrill et al. (1965) found that the utilization of lactose was high and the apparent digestion of sucrose was 84 per cent. The calves were fed a milk diet at all times and if one assumes that the lactose and sucrose by-passed any microbial action of the rumen, then the results of Morrill et al. (1965) are in extreme contrast to those previously discussed especially with regard to sucrose utilization. Morrill et al. (1965) attribute part of the disappearance of sucrose from the small intestine to bacterial activity while not discounting the possibility of digestive secretions.

There is evidence to indicate that disaccharides (maltose, sucrose, and lactose) which enter the colon are hydrolyzed and fermented by bacteria in the nonruminant (human). A human lacking invertase excretes large amounts of fatty acids in the feces following sucrose ingestion (Wilson, 1962).

No data are available on the utilization of carbohydrates caudal to the ileum. Enzyme assays indicate that the intestinal tissue in this area produces no enzymes necessary for hydrolysis of carbohydrates. Carbohydrates and other food residues which remain after microbial and

enzyme action cranial to the cecum pass into the cecum and colon where they are subjected to microbial attack (Goodall and Kay, 1965). The extent of the microbial activity, its potential, and importance to the animal have not been investigated. In a series of experiments, cellulose degradation in the large intestine has been reported as slight to 12 per cent of the total cellulose digested in sheep (Goodall and Kay, 1965). Cellulose digestion in the large intestine in cattle was 11.6 per cent of the total cellulose digested (Hale, Duncan, and Huffman, 1947).

Although there are quantitative differences in the literature concerning the ability of the ruminant to digest carbohydrates in the intestinal area, all results, with minor exceptions, show the same trend.

IV. GLUCOSE ABSORPTION IN THE SMALL AND LARGE INTESTINE

Abundant literature is available on the absorption of glucose from the lumen of the small intestine of nonruminant animals. Sherlock and Walshe (1946) observed a rise in the glucose concentration in an abdominal anastomotic vein after glucose feeding in a human subject. Proof that glucose was absorbed into the blood and transported via portal vein was provided by Kiyasu, Katz, and Chaikoff (1956) and Atkinson, Parsons, and Smyth (1957). Hendrix and Sweet (1917) concluded that the walls of both the capillaries and lymph vessels were permeable to glucose, but that the major portion of glucose was absorbed into the blood. Splanchnic blood flow in the rat is 400 ml. per hour (Reininger and Sapirstein, 1957) while flow of lymph in the thoracic duct is 0.5 ml. per hour (Shrewsbury and Reinhardt, 1952).

Although there has been some question as to the chemical form of absorbed glucose in the blood, it appears to be present predominately as glucose with only a minor portion converted to lactic acid in rats and dogs (Kiyasu and Chaikoff, 1957 and Atkinson, Parsons, and Smyth, 1957). The latter researchers indicated that 90 per cent of the glucose which disappeared from the lumen appeared in the blood as glucose while approximately 10 per cent was converted to lactic acid. The in vivo conditions which Atkinson, Parsons, and Smyth (1957) used have the advantage in that there is a good supply of glucose coming to the tissue via arterial input. In an in vitro system the only supply of glucose to the mucosa is that which is in the lumen of the intestine.

In the nonruminant animal the upper part of the small intestine has a greater capacity to absorb glucose than the lower part while the stomach and the colon absorb little (Wilson, 1962). From a calculation of mucosal surface area, Fisher and Parsons (1949) concluded that the absorption rate for glucose per unit surface area was about three times greater in the upper jejunum than in the lower ileum.

Since this thesis does not deal with the specificity of sugar absorption, that aspect of sugar utilization shall only be recognized in the scope of this paper by the fact that glucose and galactose are the most rapidly absorbed sugars, maltose and starch absorption, if any, is insignificant, and cellulose is not absorbed without degradation (Wilson, 1962).

Many investigators have shown that glucose can be absorbed against a concentration gradient (Campbell and Davson, 1948; Fisher and

Parsons, 1949; and Wilson and Wiseman, 1954). This movement against a concentration gradient, or active transport as it has become known, has been the subject of study for many researchers in the past few years. Although no complete mechanism is yet understood for this transport, some facts are known and some theories have been formed. Certain compounds do act as inhibitors and retard the absorption of glucose. Iodoacetate has been shown to reduce the absorption of glucose in the rat intestine to one-third the original rate or to approximately the same rate of absorption as fructose, mannose, xylose, and arabinose which are believed to simply diffuse across the intestinal wall (Wilson, 1962). Although iodoacetate reacts with sulfhydryl groups of many proteins and cannot be classified as a specific inhibitor, the fact that its presence does retard the absorption of glucose (and galactose) while having no effect on the absorption of arabinose, xylose, and mannose (Wilson, 1962) indicates that it does interfere either directly or indirectly with the transport mechanism which is something more than simple diffusion.

The fact that sodium and potassium ions appear necessary for active absorption of glucose gives further support of the active transport theory and some insight into the possible mechanisms involved. Riklis and Quastel (1958) demonstrated that increased concentration of potassium could increase glucose absorption. The importance of sodium on the mucosal side of the intestine has been shown to have a more dramatic effect on glucose absorption. The absence of sodium has been shown to completely stop active sugar transport (Csáky, 1960; Clarkson

and Rothstein, 1960; Csáky and Zollicoffer, 1960; and the article by Crane, Miller, and Bihler, 1961 referred to by Wilson, 1962).

No detailed investigations of quantitative glucose absorption or mechanisms of glucose absorption in the ruminant intestinal tract could be found in the literature.

Papers presented at the Second International Symposium on the Physiology of Digestion in the Ruminant and published in a book edited by Dougherty et al. (1965) give an excellent review of research in physiology of ruminant digestion.

CHAPTER III

EXPERIMENTAL MATERIALS AND METHODS

I. ANIMALS

A total of 33 yearling crossbred wethers were utilized for the complete study. Seven wethers were slaughtered and intestinal segments from the duodenum (first 20 cm. caudal to the pylorus), jejunum (middle of the small intestine), and ileum (first 30 cm. cranial to the cecum) were obtained for an assay of carbohydrases.

The remaining 26 wethers were used during the in vivo studies of glucose absorption and starch, maltose, and cellulose utilization.

The wethers, ranging from 37 to 50 kg. in body weight, were maintained on a conventional diet of 227 gm. of concentrate per day and hay ad lib., and had been wormed at the time of purchase.

II. ENZYME ASSAY

The procedure employed to assay the enzyme activities was similar to the method used by Dahlqvist (1964) for the assay of intestinal disaccharidases. Segments of the small intestine from the duodenal, jejunal, and ileal areas were obtained within 5 minutes after slaughter; the lumen was washed free of intestinal contents with cold saline taking precautions not to damage the mucosal surface. The segments were then placed on ice and taken to the laboratory where they were opened in a

manner that would expose the mucosal cells. The mucosal cells were scraped from the wall of the intestine with a microscope slide onto a second microscope slide and weighed wet. The removal of the mucosal cells from the intestinal segment and the weighing were done at room temperature and required approximately 5 minutes for completion. The cells were washed from the slide with cold saline (4 ml. per gm. of mucosal cells) into a Thomas homogenizing tube and homogenized for 1 minute. The homogenate was then transferred to a centrifuge tube and the homogenizing tube was rinsed with cold saline (4 ml. per gm. of mucosal cells). The homogenate was centrifuged at 900 x g. in a cold (2° C.) centrifuge for 10 minutes to separate the cell residue from the saline extract. The supernatant was pipetted into polyethylene bottles and placed in an ice bath.

Solutions of 0.056 M. maltose, lactose, sucrose, and cellobiose were prepared in 0.1 M. maleate buffer. A soluble starch solution was prepared using a quantity of starch equivalent to the quantity of maltose used for the 0.056 M. maltose solution.

Maleate buffers of pH 6.0, 6.5, and 7.0 were prepared by dissolving 1.16 gm. of maleic acid in 15.3, 17.8, and 19.1 ml. of 1 N. sodium hydroxide, respectively, and diluting to 100 ml. with distilled water. Lactose was dissolved in the pH 6.0 buffer, maltose in the pH 6.5 buffer, and sucrose, cellobiose, and starch in the pH 7.0 buffer.

Since all substrates yielded at least one molecule of glucose per molecule of substrate hydrolyzed, the glucose yield was used to determine the enzymatic activity.

Glucostat glucose reagent (Worthington Biochemical Company) was used to determine the glucose produced. The crude glucostat is not pure glucose oxidase and contains contaminant enzymes which will hydrolyze certain disaccharides such as maltose (Dahlqvist, 1964). These contaminants make the glucose assay in the presence of some disaccharides unreliable. Tris(hydroxymethyl)aminomethane inhibits the disaccharidase activity without interfering with the glucose oxidase reagent (Dahlqvist, 1964).

One set of "glucostat x 4" contains a vial of the enzyme (glucostat) and a vial of the color developing reagent (chromogen). The Tris-glucose oxidase reagent was prepared by dissolving the contents of the glucostat vial in 10 ml. of the Tris buffer (61.0 gm. of Tris in 85 ml. of 5 N. HCl made up to a liter with distilled water) and dissolving the contents of the chromogen vial in 2 ml. of polyvinylpyrrolidinone and 3 ml. of Tris buffer, mixing the two solutions and diluting with the Tris buffer solution to 400 ml.

A standard glucose solution was prepared by dissolving 50 mg. of glucose and 1.4 gm. of benzoic acid in distilled water and diluting to 500 ml.

The assays for maltase, lactase, sucrase, cellobiase, and amylase were performed by transferring 0.1 ml. of the substrate solution and a small drop of toluene into a 7 x 120 mm. test tube and placing the tube in a water bath at 37° C. One-tenth ml. of saline extract from the mucosal cells was added and the mixture was incubated for 1 hour. At the end of the incubation time, 0.8 ml. of distilled water was added and

the tube was immediately placed in a boiling water bath for 2 minutes to stop the reaction and then cooled in an ice bath. Blanks were prepared in the same manner using distilled water in place of the substrate and all assays were performed in triplicate.

To determine the glucose produced from the carbohydrase activity, 3.0 ml. of Tris-glucose oxidase solution was added to 0.5 ml. of the incubated saline extract-substrate mixture in a 16 x 150 mm. tube and this mixture was incubated for an hour at 37° C. At the same time 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 ml. of the standard glucose solution and distilled water to a combined volume of 0.5 ml. were incubated for an hour at 37° C. with 3.0 ml. of Tris-glucose oxidase reagent. These tubes contained 0, 10, 20, 30, 40, and 50 µg. of glucose, respectively.

The optical density of the unknowns and standard solutions resulting from the color development was determined on a Beckman DU spectrophotometer at 420 mµ. The readings for the unknowns were compared to the readings for the standards, converted to µg. of glucose produced, and then to micromoles of substrate hydrolyzed. Starch was converted to a maltose equivalent in computing enzyme units (arbitrary). One unit of enzyme activity is defined following the recommendations made by the Joint Sub-Commission on Clinical Enzyme Units of the International Unions of Biochemistry and of Pure and Applied Chemistry (Dahlqvist, 1964), as that amount of activity that will hydrolyze 1 micromole of substrate per minute.

Since it was not always convenient to assay the activity in the saline extract immediately, the effects of storage on the activity

was investigated. The saline extract was divided into three equal parts and placed in polyethylene bottles and the bottles were sealed. One part of the saline extract was stored at 4° C., another part was stored at -20° C., and the third part was further divided into four equal fractions which were also stored at -20° C. Each week for 4 weeks the saline extract stored at 4° C. and the undivided fraction stored at -20° C. were removed, assayed, and returned to their respective storage places. Each week for 4 weeks one subsample from the third fraction was removed from the freezer, assayed, and discarded.

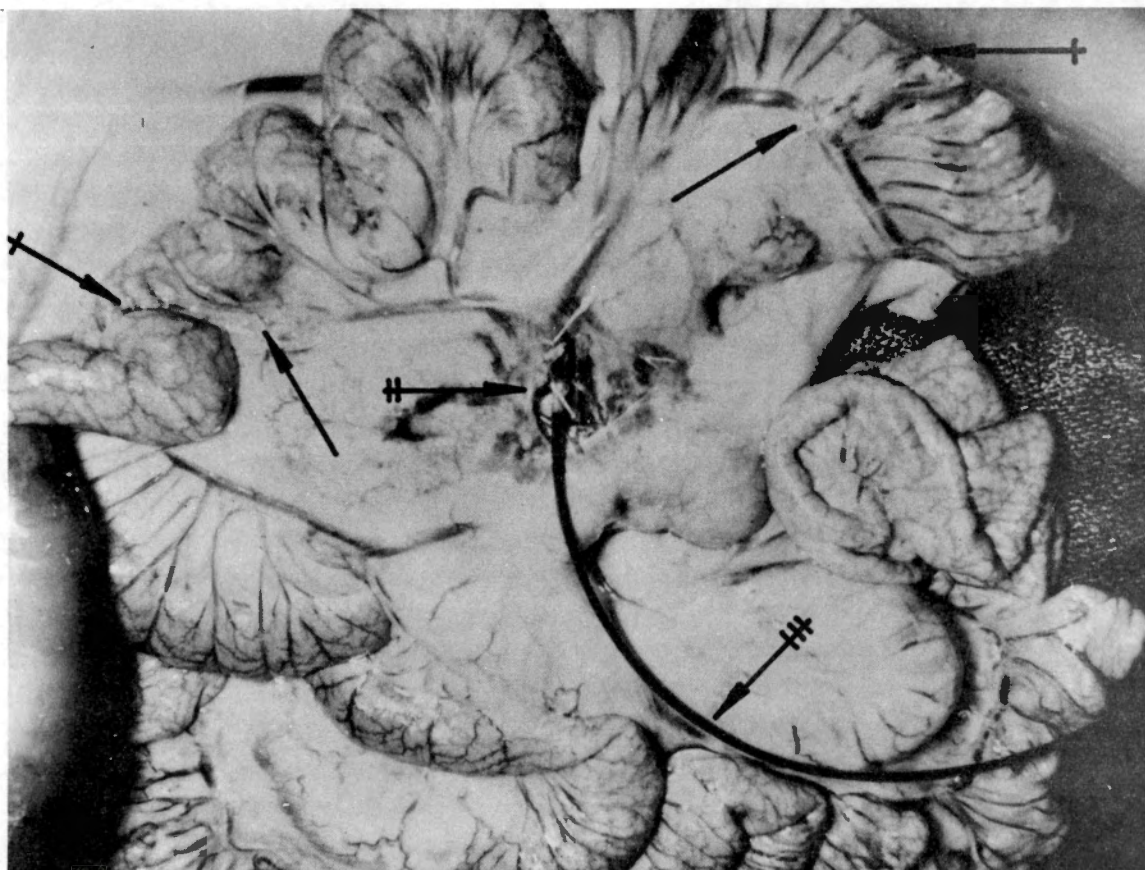
III. HYDROLYSIS AND ABSORPTION

Surgical preparation. The wethers used for the carbohydrate utilization and absorption study were removed from feed approximately 12 hours prior to the experiment. The sheep were anesthetized with either diabutal or electrical anesthesia. An intravenous injection (gravity) of a 0.154 M. sodium chloride solution containing glucagon-free-insulin (Eli Lilly and Co.) was administered through the right jugular vein during the preparation and experimental period. The insulin entered the circulation at the rate of 0.75 units per kg. body weight per hour.

Once the animal was anesthetized and the injection started, a 20 to 30 cm. incision was made right of midventral and the area of the intestines desired for the study was exposed. As much of the intestinal tract as possible was placed back inside the abdominal cavity to prevent shock and loss of heat and fluid. The sections of intestine to be used were then flushed with warm saline by placing a hypodermic needle into

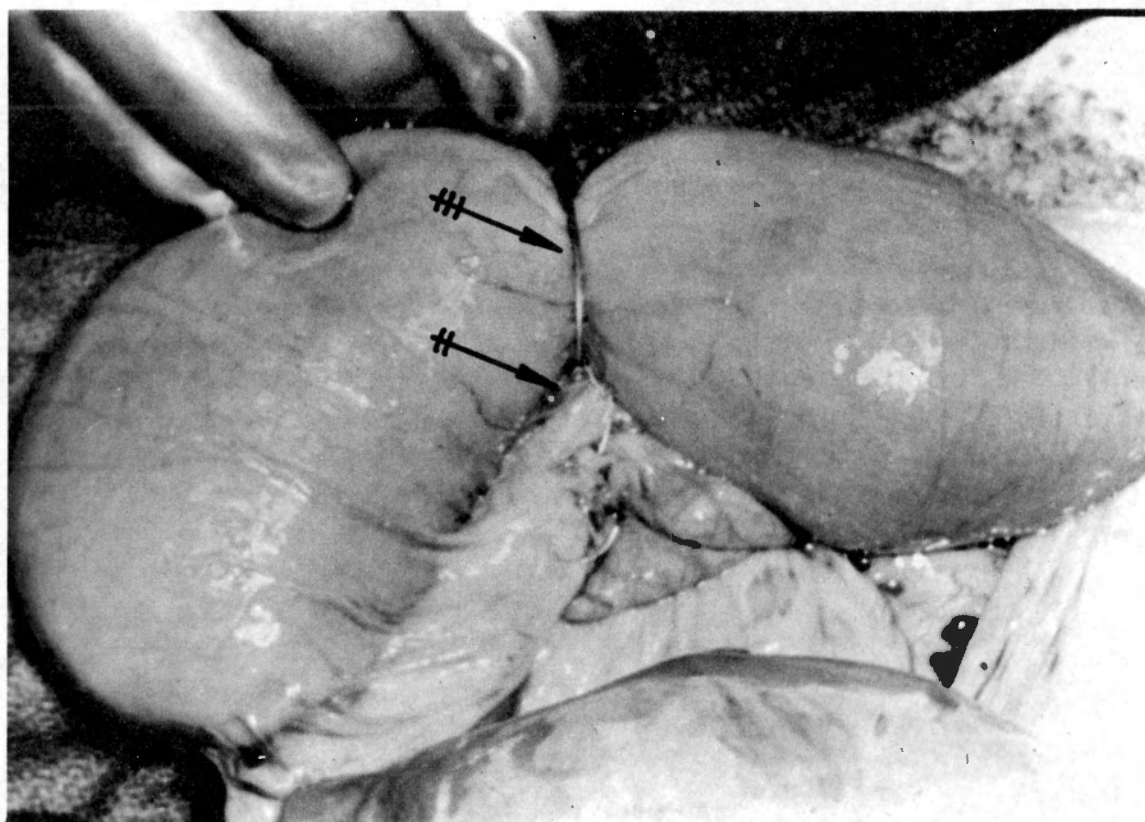
the lumen. Isolated segments were prepared by placing ligatures 75 cm. apart along the flushed section of intestine. The mesenteric vein which carried all the blood from the isolated segment was selected for cannulation, separated from its associate artery, and two ligatures were placed around the vein but not drawn tightly. All veins which did not carry blood from the isolated segment, but which did join the mesenteric vein below the point selected for cannulation, were ligated to prevent the blood from entering the vein selected for cannulation.

The animal was then heparinized with an injection of 10 cc. of "panheprin" (1,000 U.S.P. units per ml., Abbott Laboratories) into the right jugular vein. The ligature on the mesenteric vein distal to the intestine was tied tightly and a small incision was made in the vein between the tightened ligature and the remaining loose ligature. A cannula made from PE 200 polyethylene tubing, flared on one end by heating, was placed into the vein through the incision. After passing through the incision, the flared end was passed through the lumen of the vein and past the loose ligature. The ligature was tightened to hold the cannula in place and to prevent leakage of blood around the cannula. This preparation made it possible to collect all the blood and only the blood draining the isolated intestinal segment. All preparations were similar, but varied in position along the intestinal tract. Preparations were not possible using this technique in the duodenum due primarily to the lack of a large common mesenteric vein, anatomical location, and the presence near the cranial portion of a double venous system. Figures 1 and 2 illustrate the preparations in the small



- Ligatures isolating mesenteric vein draining ligated intestinal segment.
- +—●— Ligatures isolating intestinal segment.
- ==—●— Point of cannula entry into mesenteric vein.
- ≡—●— PE 200 polyethylene tubing (cannula).

Figure 1. Surgical preparation of the small intestine.



— Point of cannula entry into mesenteric vein.

— PE 200 polyethylene tubing (cannula). Beneath cannula lies ligature isolating blind end of cecum from the rest of the cecum.

Figure 2. Surgical preparation of the cecum.

intestine and the cecum. The principle was the same in the colon where a vein in the coil was cannulated.

Normally three preparations were made adjacent to each other and blood collections were made simultaneously from each using two for absorption studies and one for a blank.

At the conclusion of the experiment with each sheep, the animal was sacrificed with an intravenous injection of 30 to 50 ml. of Equi-Thesin (Jensen-Salsberry, Co.).

Experimental procedure. Four observations for the utilization of maltose, glucose, and starch substrate solutions were made in the jejunum and ileum, each observation representing a different sheep. Four observations for maltose and starch were also made in the cecum while two observations were made in the cecum and colon with glucose. Three observations were made on the effects of ouabain and iodoacetate on glucose absorption in the jejunum. Two observations were made on the effects of ouabain and iodoacetate on glucose absorption in the ileum and one observation was made on the effect of iodoacetate on glucose absorption in the cecum. Six observations were made on the degree of cellulose utilization in the cecum and two observations with cellulose were made in the jejunum and ileum.

Substrate solutions of glucose, maltose, and starch were made isosmotic with sodium chloride and potassium chloride solutions. Glucose substrate solution was made by combining three parts potassium chloride solution (12.8 gm. per liter) with two parts glucose solution

(54 gm. per liter) and five parts sodium chloride solution (10 gm. per liter). Maltose substrate solution resulted from combining three parts of the same potassium chloride solution with two parts maltose solution (54 gm. per liter) and five parts sodium chloride (11.8 gm. per liter). Starch substrate was made by combining three parts of the potassium chloride solution with two parts starch suspension (54 gm. per liter) and five parts sodium chloride solution (13.8 gm. per liter). The osmotic pressure of all three substrate solutions was equivalent to 320 milliosmols or isosmotic with sheep blood.

Ten ml. of a substrate solution was injected into the lumen of the isolated segment used for the absorption or hydrolysis and uptake study of that particular substrate. One hundred microcuries of uniformly labeled C-14 glucose was injected simultaneously with the glucose substrate solution into the intestinal segments used to study glucose absorption.

Uniformly labeled C-14 cellulose was placed into the isolated intestinal segments, which had not been flushed out with saline, by making a small incision in the segment, placing the cellulose in the lumen of the intestine, and closing the incision with a purse string suture. The segment was not flushed so that any cellulytic micro-organisms present would have an opportunity to degrade the cellulose.

After the substrates were placed in the isolated segments, blood from the cannulated veins was collected continuously for 30 minutes, allowing 3 minutes for each sample. One-half ml. of ouabain (7×10^{-2} M.) and 0.5 ml. of iodoacetate (4×10^{-2} M.) were injected into the lumen of

the segments containing glucose in five sheep and in these cases collections of blood were continued as long as an hour.

Analytical procedure. The volume and hematocrit of the blood collected were recorded and the plasma was separated from the red cells by centrifugation at 1,400 x g. for 15 minutes. Plasma protein was precipitated by placing 1 ml. of plasma in a 16 x 150 mm. test tube, adding 5 ml. of water, 2 ml. of 5 per cent zinc sulfate, and 2 ml. of 0.3 N. sodium hydroxide. The mixture was shaken on a "Vortex" mixer to prevent a colloidal suspension and to insure complete precipitation of the protein. The precipitate was filtered out with Whatman number 42 or 50 filter paper.

The absorption of glucose was determined by either "Glucostat" analysis or by radiochemical analysis for glucose in the plasma. The glucose in the plasma from the isolated segment which contained the injected glucose solution was compared to the glucose level in the plasma from the blank segment which contained only a sodium chloride solution. The difference was a measure of the glucose absorbed. Since the end product of maltose and starch hydrolysis is glucose, the increased plasma glucose level was also used to determine the hydrolysis and uptake of these two carbohydrates.

Plasma glucose was determined by placing 1 ml. of the filtrate, after the plasma proteins were precipitated, into a 16 x 150 mm. test tube and adding 9 ml. of glucostat reagent. The mixture was incubated for 10 minutes at room temperature. The reaction was stopped by adding

2 drops of 4 N. HCl. The glucostat reagent used for this determination was dissolved in distilled water since any disaccharides present were not in a quantity large enough to interfere with the glucose determination. The color developed during the incubation was compared, on a spectrophotometer, to the color developed from the incubation of glucose standards.

The second method for determining the glucose absorption and the only method used for determining cellulose utilization was a radiochemical analysis. The scintillator fluid used for determining radio-carbon consisted of 4.0 gm. of PPO, 0.1 gm. of POPOP, 75 gm. naphthalene, and 150 ml. of bis-(2 ethoxyethyl)ether made up to a liter with dioxane. One ml. of the plasma filtrate was pipetted into each scintillator vial and 14 ml. of scintillator fluid was titrated into each vial. All samples were prepared in duplicate and counted in a three channel Nuclear-Chicago liquid scintillation detector.

Radiostandards were prepared by adding a known quantity of C-14 labeled glucose to a known volume of plasma and following the same analytical procedure used for the unknowns. This method of preparing standards eliminated the necessity of a correction for any dilution, loss on the filter paper, and quenching. All activity was converted to per cent dose, then converted to milligrams of glucose absorbed. Results of the glucostat analysis were also expressed in terms of milligrams of glucose absorbed.

The identity of the major compounds containing C-14 in the plasma was determined by chromatographing the filtrate on paper and making

autoradiograms of the C-14 distribution. One hundred μ l. of the filtrate, 25 μ l. at a time, was placed on Whatman number 3 mm chromatography paper strips and irrigated three successive times with a mixture of 3 parts butanol, 1 part pyridine, and 1.5 parts water, drying the strips between irrigations. The strips were dried and placed on X-ray film for 16 days. The film was then developed to determine the location of the C-14 labeled materials. The identity of the C-14 labeled material was determined from the location of known material identified on paper strips by color development resulting from spraying with dinitrosalicylic acid and drying. After drying, the paper was heated at 105° C. for 5 minutes to develop the brown spot indicating the location of the known substance.

C-14 labeled cellulose was assayed for water soluble C-14 impurities resulting from radiolysis. An analysis of a water extract was made for glucose using the glucostat reagent and 100 μ l. was placed on chromatography strips, irrigated twice with the same solvent used to separate C-14 labeled materials from the plasma extract and autoradiograms were made on X-ray film.

CHAPTER IV

RESULTS AND DISCUSSION

I. CARBOHYDRASE ACTIVITY IN THE INTESTINAL MUCOSA

The hydrolysis and uptake of any disaccharide or larger molecular weight carbohydrate in the small intestine is primarily dependent on the following: the number of microorganisms present that can degrade carbohydrates to some end product(s) which can be absorbed and utilized by the animal body; the active enzymes present in the lumen of the intestine as a result of microbial release, pancreatic secretion, or desquamated intestinal epithelial cells that will hydrolyze carbohydrates; and the carbohydrases present in the mucosa cells of the intestine. These are all important factors determining the degree of utilization of any carbohydrate present in the intestinal area.

The mucosa of the jejunum in the mature sheep contained the greatest concentration of all enzymes assayed (Figure 3) and the duodenum contained the least of all with the exception of lactase activity. The maltase activity was greater in each of the areas than the activities of the other enzymes in the same area. Amylase was the next most abundant enzyme present in the jejunum and ileum. The means were calculated from observations of the three areas in seven sheep. The probabilities of significance and the mean values are listed in Table IV of the Appendix. From the activities shown in Figure 3 it is

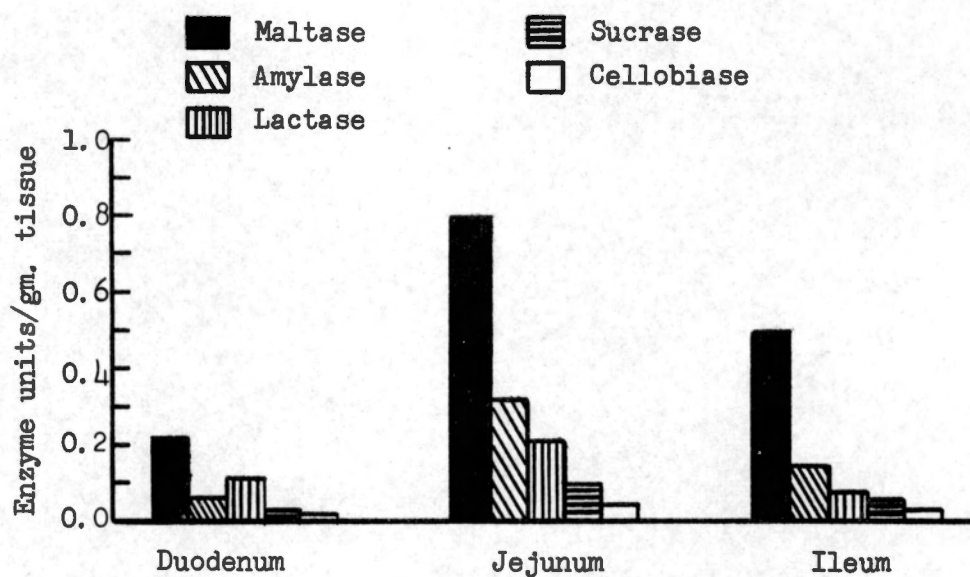


Figure 3. Relative enzyme activity in duodenum, jejunum, and Ileum of seven mature sheep.

apparent that, from the standpoint of intestinal carbohydrases only, the hydrolytic enzymes in the jejunum make this area of the intestine more important than the duodenum and ileum for the hydrolysis of intestinal carbohydrates. It must also be recognized that a relatively large amount of maltose as well as some glucose is produced by the amylase activity and that the amylase assay and the complete hydrolysis of starch by the animal are both dependent on maltase activity. However, it is obvious from the quantity of maltase present that it was not a limiting factor in the analysis of amylase. The importance of pancreatic amylase, which was not assayed, for starch hydrolysis to maltose cannot be denied. Since the pancreatic duct enters the small intestine caudal to the point of duodenal enzyme assay, it is not surprising to find the greater concentrations of maltase caudal to the opening of the duct. That area of the small intestine would contain the greater quantity of maltose resulting from starch hydrolysis. One possible physiological explanation for the comparatively low enzyme activity in the duodenum is the fact that the pH is more acid in this area than it is farther down the tract due to abomasal emptying. This lower pH may destroy a portion of the activity. A less superficial possibility would be differences in rates of biosynthesis of the enzymes. Since the assay was made in the cranial 20 cm. of the duodenum, this may not be a true picture of the activity in other portions of the duodenum.

The higher activities of maltase and amylase compared to lactase in the jejunum and ileum observed here are in agreement with the work of Dollar and Porter (1957 and 1959) and Walker (1959). The enzyme

activities found in the jejunum and ileum of these mature sheep are much lower (0.8 unit compared to 13-54 units for maltase, 0.1 unit compared to 6-17 units for sucrase, etc.) than the activities of the jejunum and ileum of humans determined by Dahlqvist (1964). The enzyme activities from the sheep's small intestine are also lower than the activities reported for mucosal cells of pigs (Walker, 1959) and agree with Walker's conclusion that the mucosal cells of pigs contain more activity than the mucosal cells of sheep. If the sucrase activity in the mucosa of sheep and calves is comparable, then the disappearance of sucrose from the small intestine of calves (Morrill, 1965) must have been due to something other than mucosal sucrase. Morrill's results are questionable since he observed no increase in circulating blood reducing sugars though the apparent digestibility of the sucrose was 84 per cent.

Although the extent of adaptation of carbohydrase production in the intestine resulting from changing substrate levels is not clear (Wilson, 1962), one factor which may influence the increase in maltase and amylase and the decrease in lactase with increasing age in both ruminants and nonruminants is an adaptation of carbohydrase biosynthesis due to increased or decreased substrate levels in the intestine. This explanation has support from the fact that as the milk diet is replaced with a concentrate diet the lactase level falls and the levels of other enzymes rise. Another factor lending support to the theory is that starch, maltose, and sucrose are found in much higher levels in the intestinal tract of the nonruminant than the ruminant and the corresponding hydrolytic enzyme activities are higher in the nonruminant.

Such a biosynthetic adaptation might help to explain the lower levels of enzyme activities in the ileum compared to the jejunum. The quantity of carbohydrates reaching the ileum would be less than in the jejunum, resulting in less synthesis of hydrolytic enzymes.

The low levels of carbohydrases present in the small intestine of the mature sheep makes this animal a useful tool for a study of the effect of high substrate levels on the adaptation of intestinal carbohydrase activities.

Since it might not always be convenient to assay the carbohydrases immediately after preparation, it was of interest to determine the stability of the activities under simple, convenient storage conditions. Storage of the saline extract in sealed polyethylene bottles at 4° C. (Table I) for a week had little effect on all activities except lactase. However, between the first and second week of storage the activities of the other enzymes began to drop rapidly and at the end of the fourth week very little of the original activity was left. Freezing the enzymes in the saline extract decreased the original activity by nearly half and although continuous freezing (Table II) and repeated thawing and refreezing (Table III) decreased the activity some, it was the initial freeze that caused the greatest reduction in carbohydrase activity. Although there are other possibilities for the preparation of carbohydrases for storage, these data show that the most reliable assays are made on freshly prepared extracts of the mucosal cells.

TABLE I
EFFECT OF STORAGE AT 4° C. ON ACTIVITY
OF CARBOHYDRASES IN SOLUTION

Enzyme	Weeks after preparation				
	0	1	2	3	4
	% of original activity				
Maltase	<u>100.0</u>	<u>94.1</u>	<u>74.4</u>	<u>57.0</u>	20.4
Lactase	100.0	<u>39.4</u>	<u>34.1</u>	<u>16.3</u>	0.0
Sucrase	<u>100.0</u>	<u>91.5</u>	<u>11.7</u>	0.6	0.0
Cellobiase	<u>100.0</u>	<u>100.0</u>	<u>56.0</u>	<u>27.1</u>	<u>17.5</u>
Amylase	<u>100.0</u>	<u>99.0</u>	<u>82.0</u>	<u>62.8</u>	<u>34.8</u>

NOTE: Means underlined are not significantly different $P < .05$.

TABLE II
EFFECT OF CONTINUOUS FREEZING ON ACTIVITY
OF CARBOHYDRASES IN SOLUTION

Enzyme	Weeks after preparation				
	0	1	2	3	4
% of original activity					
Maltase	100.0	<u>61.6</u>	<u>47.9</u>	<u>42.9</u>	<u>36.3</u>
Lactase	100.0	<u>40.4</u>	<u>33.7</u>	<u>22.8</u>	<u>10.6</u>
Sucrase	100.0	<u>45.0</u>	<u>7.7</u>	<u>31.6</u>	<u>22.0</u>
Cellobiase	100.0	<u>61.6</u>	<u>69.0</u>	<u>29.1</u>	<u>25.0</u>
Amylase	100.0	<u>63.3</u>	<u>62.0</u>	<u>67.0</u>	<u>48.1</u>

NOTE: Means underlined are not significantly different $P < .05$.

TABLE III
EFFECT OF REPEATED THAWING AND REFREEZING ON
ACTIVITY OF CARBOHYDRASES IN SOLUTION

Enzyme	Weeks after preparation				
	0	1	2	3	4
	% of original activity				
Maltase	100.0	<u>61.7</u>	<u>47.7</u>	<u>33.0</u>	<u>28.4</u>
Lactase	100.0	<u>40.4</u>	<u>32.7</u>	<u>28.4</u>	<u>13.5</u>
Sucrase	100.0	<u>45.0</u>	<u>10.4</u>	<u>23.0</u>	<u>1.6</u>
Cellobiase	100.0	<u>61.6</u>	<u>32.0</u>	<u>39.0</u>	0.0
Amylase	100.0	<u>63.3</u>	<u>54.5</u>	<u>52.2</u>	<u>41.3</u>

NOTE: Means underlined are not significantly different $P < .05$.

II. EFFECTS OF ANESTHESIA AND SURGERY

Blood glucose levels of the sheep used for the in vivo studies increased continuously from the initiation of anesthesia to the completion of the study on each animal (Figure 4). Although the effects of the high level of glucose in the blood on glucose absorption from the intestine was not investigated, a level of glucose in the blood which approached normal physiological conditions was considered advantageous. An attempt was made to lower the level of circulating glucose by eliminating the epinephrin through adrenalectomy. Although adrenalectomy did slightly lower the level of circulating glucose during the experimental period (Figure 4), it was obvious that some factor(s) played a much greater role in increasing the blood glucose level than epinephrin. Galansino et al. (1960) suggested that more than one type of hyperglycemic substance may be produced in the area supplied by the pancreatic artery; one substance like serotonin or the catecholamines which can be blocked by dihydroergotamine and another type like glucagon which is not blocked by dihydroergotamine. Since removal of the pancreas was considered too intricate for the number of animals involved in this study, insulin was used to alleviate the hyperglycemic condition. An insulin injection of 0.75 units per kg. body weight per hour was found to hold the blood glucose to a near normal level during the experimental period (Figure 4). The effects, if any, of insulin injection on glucose absorption appears to be slight. Sols, Vidal, and Larralde (1948) obtained a rise in the glucose absorption rate of 8 per cent in the

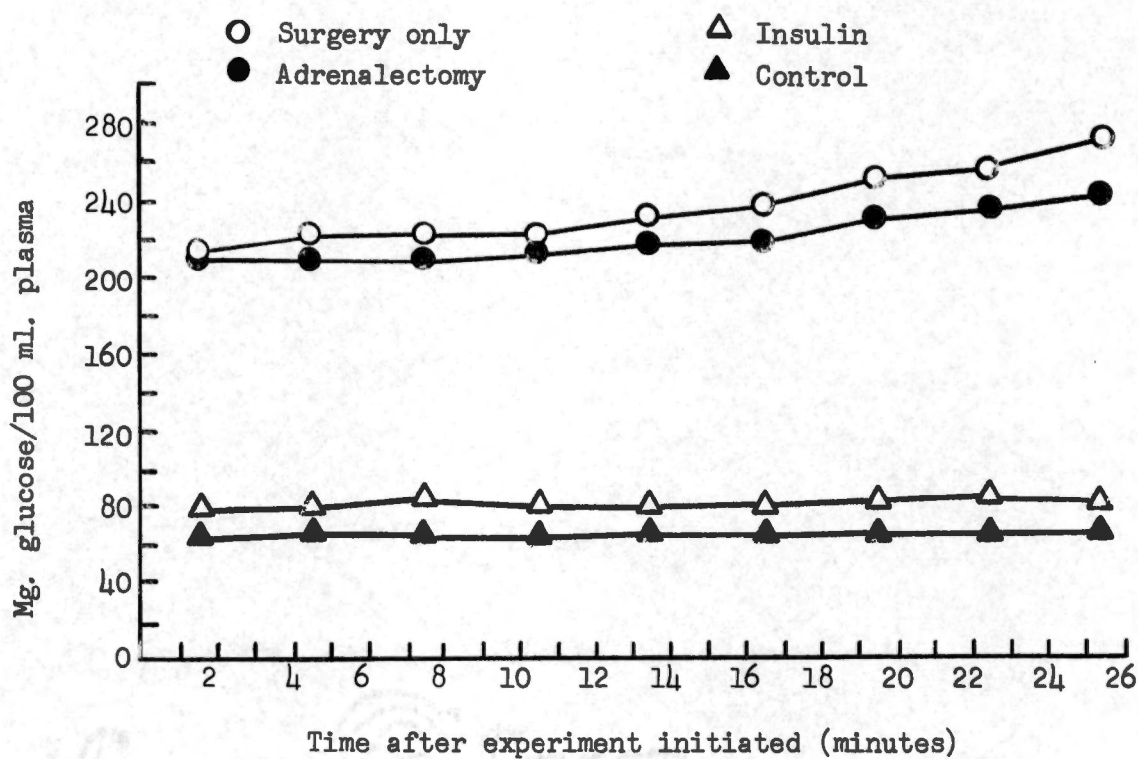


Figure 4. Effect of adrenalectomy and insulin treatment on hyperglycemic conditions resulting from anesthesia and surgical shock.

first 30 minutes after a single injection of two units of insulin per 100 gm. body weight in rats. Any effect of insulin injection on glucose absorption during this investigation was assumed to be quite small since only 0.15 units of insulin per 100 gm. body weight was injected during the entire experimental period.

Surgery and the duration of the experiment had little effect on the rate of blood flow from the various segments (Figure 5). The intestinal area used in expressing the blood flow in Figure 5 and for absorption comparisons in later data was computed from serosal measurements and was not a measurement of mucosal surface area. Although no absolute measurements were made, the temperature of the intestines during the experiment appeared to have the greatest effect on the flow rate of the blood. For this reason the intestines were constantly bathed with warm saline throughout the experiment. At no time during the blood collection periods did the color of the intestines indicate an anoxic condition and the PE 200 tubing was considered sufficient to maintain normal blood drainage.

Blood flow per unit serosal area was less from the small intestine than from the colon and cecum. No difference was observed in the flow from the jejunum and ileum. Flow from the cecum decreased more during the experimental period than from any other section of the intestine. The cecum was the most difficult section to keep warm and this is probably the reason for the decreased flow. Individual data with the standard deviations appear in Tables V and VI of the Appendix.

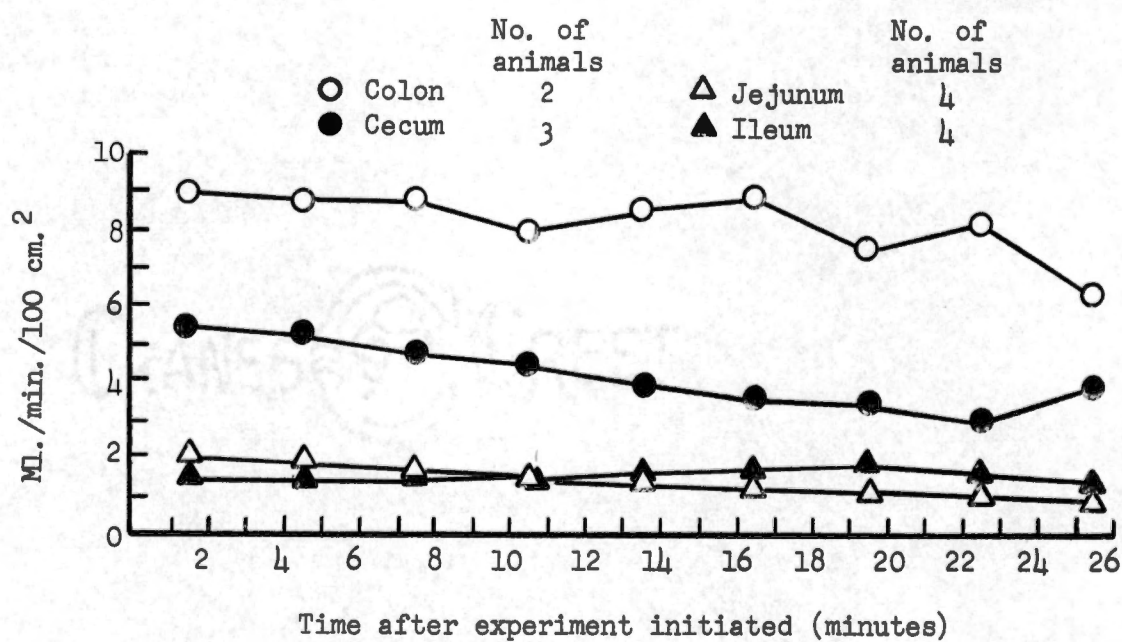


Figure 5. Plasma flow from various parts of the intestinal tract during experimental period.

III. GLUCOSE ABSORPTION

Direct collection of the blood flowing from the isolated intestinal segments had the advantage over blood sampling by jugular puncture employed by Huber et al. (1961) in that the glucose absorbed did not reach the liver. Some of the glucose in the blood entering the liver from the intestinal area after glucose absorption is converted to glycogen thus lowering the blood glucose level. Sampling from the jugular would lead to values lower than the actual glucose values in the blood draining the intestinal area. Further advantages of this method are that a quantitative evaluation of the absorption may be made; there is no dilution from blood draining other areas of the body; and the glucose level of the arterial blood supplying the intestinal area remains constant. Collections made simultaneously from the isolated segment used as the blank made it possible to follow the glucose level of the circulating blood and obtain the amount of glucose absorbed by difference. Excitement of an unanesthetized animal can result in rises in blood glucose which can lead to unreliable results using the technique of Huber et al. (1961).

Total absorption from the intestine depends on the amount of substrate present in the lumen, the loading capacity of the blood draining the intestine, the absorptive area, and the rate of passage of the absorbed material across the intestinal wall. The absorption of glucose from the jejunum, ileum, cecum, and colon (Figure 6) indicates that the loading capacity of the blood was not reached in any of

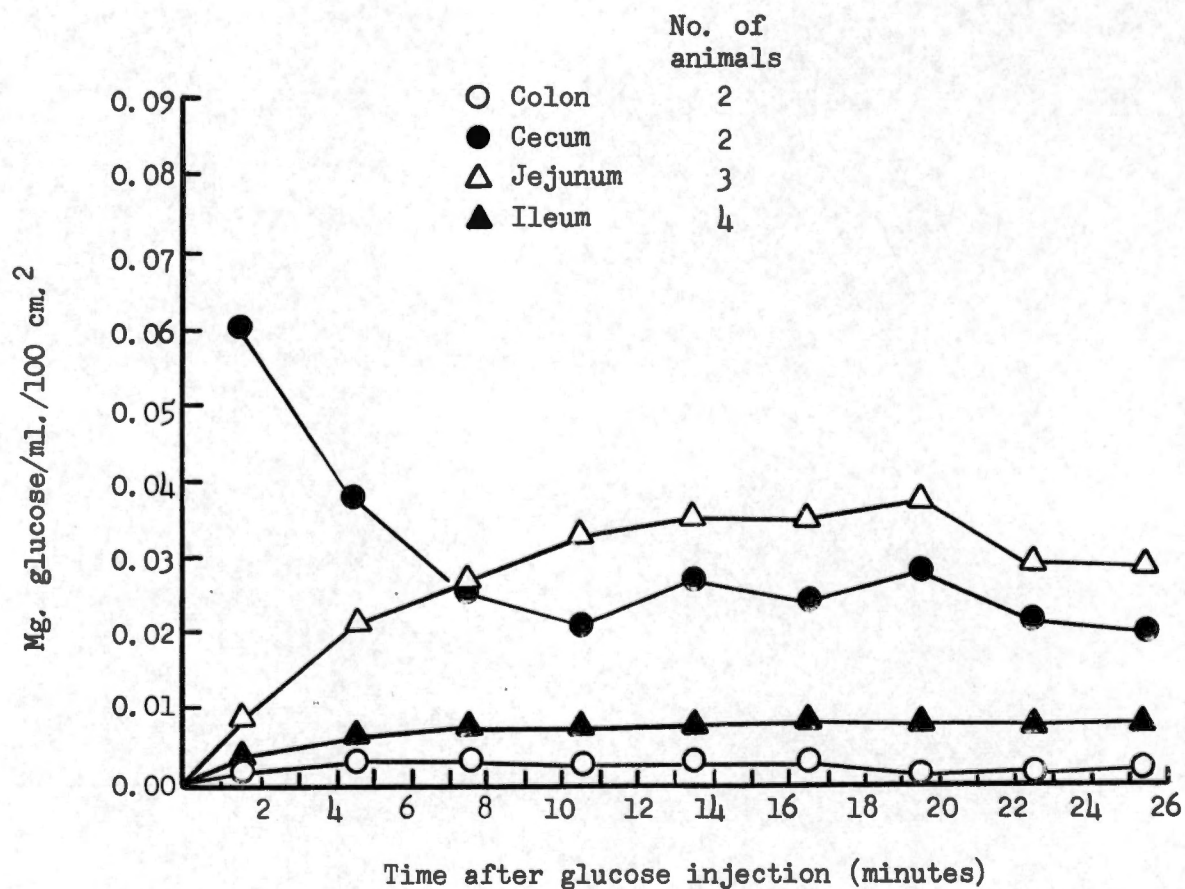


Figure 6. Plasma glucose load from various parts of the intestinal tract during glucose absorption.

the areas. A plateau in the blood glucose concentration was reached approximately 7 minutes after the substrate was injected into the intestine. Glucose concentration curves from the ileum, jejunum, and colon differed in magnitude, but were similar. The concentration curve for the blood draining the cecum was almost a mirror image of the other three curves. In the blood draining the cecum the highest concentration was in the first collection or 1.5 minutes after the injection of the glucose. At this time the glucose concentration in the blood was higher than any other time during any collection. For this reason the plateaus were not considered to be at the loading capacity of the blood.

Differences in the sizes of the isolated segments used were corrected by expressing the absorption in terms of 100 cm.^2 of serosal area. By injecting a constant quantity and concentration of glucose (108 mg. in 10 ml. of sodium chloride and potassium chloride), expressing the absorption per unit area, and not approaching the loading capacity of the blood, the absorption rate of glucose for the different areas of the intestinal tract was calculated (Figure 7). The concentration of the glucose in the injected solution was 1,080 mg. per 100 ml. compared to about 50 mg. per 100 ml. in the circulating blood of the sheep. Therefore, simple diffusion was in the direction of the blood. A comparison of the values for the rates of absorption longer than 7 minutes after the injection of the glucose solution should give the most reliable information because the injected solution has had time to become evenly distributed throughout the isolated segment.

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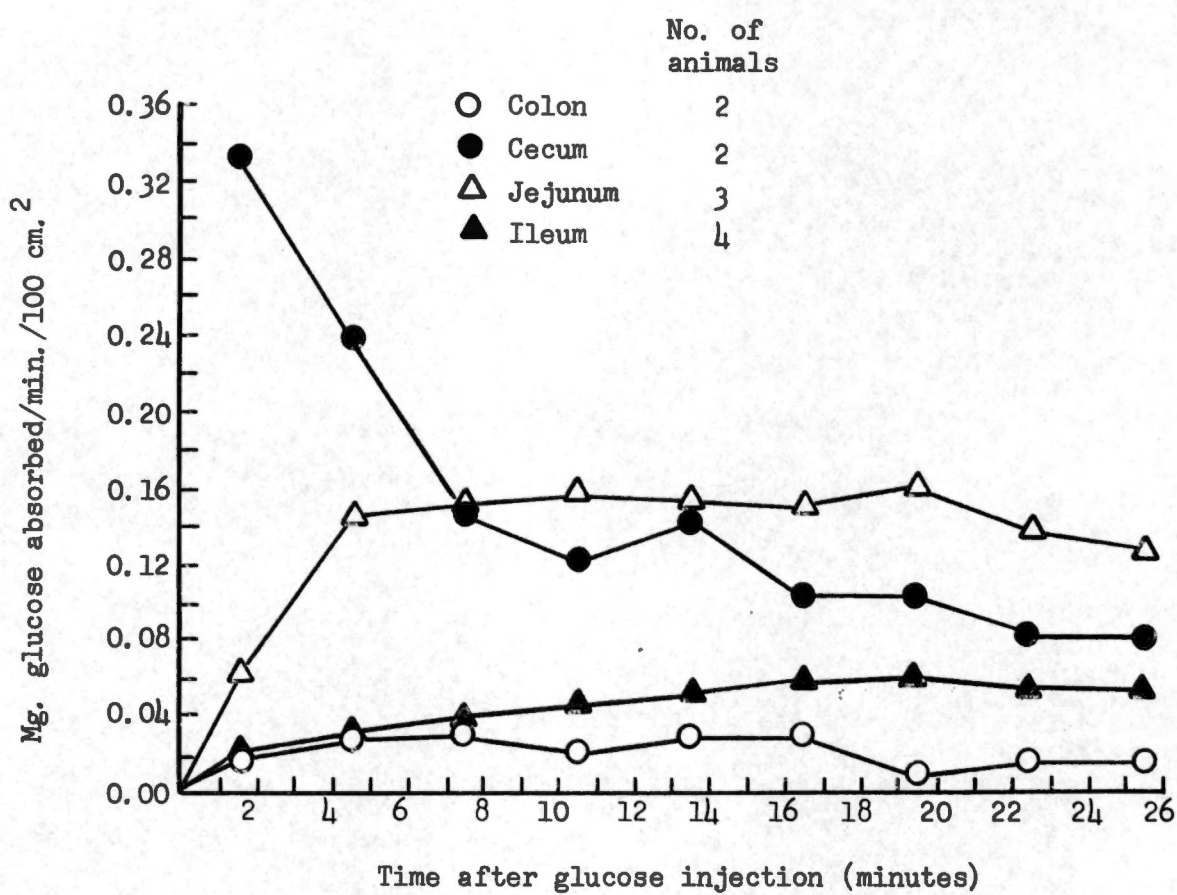


Figure 7. Absorption rates for glucose from various parts of the intestinal tract.

The rate of glucose absorption from the jejunum was much faster than the rate in any of the other segments (Figure 7, page 41). Fisher and Parsons (1949 and 1953) demonstrated that the jejunum of rats absorbed glucose at a faster rate than the lower ileum which agrees quite well with the results of this study. The rate of glucose absorption from the jejunum was 2.5 to 3 times greater than that from the ileum. These figures are in good agreement with those of Fisher and Parsons (1949) who calculated that the rate of glucose absorption per unit area of mucosal surface was three times greater in the jejunum than in the ileum of the rat. Since the absorption rates in the present study were not based on mucosal surface area, a discrepancy between the data obtained and that of Fisher and Parsons (1949) could result if the mucosal surface area per unit of serosal area in the jejunum is different from that in the ileum. Cross-sectional slides of the jejunum and ileum indicate that the villi in the jejunum are considerably longer than those located in the ileum; however, no attempt was made to calculate a mucosal surface area.

The rate of absorption of glucose from the colon was less than from the other sections investigated. This observation agrees well with the statement that the cranial part of the small intestine has a greater capacity to absorb glucose than the caudal portion while the colon absorbs little (Wilson, 1962). Since the mucosal surface of the cecum and the colon look histologically quite similar in cross section, it is surprising that the rate of absorption was much higher in the cecum. These data partially agree with early results using ligated intestinal

segments of 8-month-old calves that indicated glucose absorption was greater from the jejunum, but less from the cecum than the ileum (Larsen et al., 1956). The magnitude of the results of Larsen et al. seems questionable in that a plateau in the rate of absorption over an 80-minute period was never reached. The increase in absorption rate for the first 20 minutes was practically the same from the three areas, then the rate continued to increase in the jejunum, remained about constant in the ileum, and decreased in the cecum during the rest of the experimental period employed by Larsen et al. Results of the current investigation indicate a decrease in the rate of absorption in the cecum for the first 7 minutes and then a constant rate for the next 20 minutes while the rates of absorption from the other three areas also remained about constant during the same 20-minute period.

Differences in the absorption rates could be a result of different diffusion constants due to tissue thickness or location of capillary systems, differences in mucosal surface areas, differences in permeability of the tissues, or differences in active transport rates. No attempt was made to compute diffusion constants or mucosal surface areas, but two inhibitors were employed in an attempt to determine the degree of absorption due to some mechanism other than simple diffusion.

Ouabain ($C_{29}H_{44}O_{12} \cdot 8H_2O$) has been shown to inhibit active sodium-potassium exchanges (Daniel and Robinson, 1960). It appears to act directly on the ion-exchange mechanism and not on metabolism. Since sodium has been shown to be necessary for the active transport of glucose, the effects of an ouabain solution injected into the lumen of

the intestine were determined (Figure 8). The 7×10^{-2} M. solution was 1,000 to 10,000 times more concentrated than solutions used by other researchers to inhibit sodium-potassium exchanges in red blood cells, cardiac tissue, and uterine tissue. The fact that ouabain failed to interfere with glucose absorption could result from a number of possibilities: (1) the ouabain solution may not have been the proper concentration to effect sodium-potassium exchange in vivo; (2) the sodium concentration in the lumen of the intestine may have been too great to give a net effect from the presence of ouabain; (3) ouabain may not interfere with the sodium-glucose mechanism responsible for glucose absorption; or (4) sodium may not be necessary for glucose absorption in the intestine of the sheep. The latter possibility seems improbable due to the experimental evidence from other species. Of the above possibilities 2 and 3 seem to be the most likely explanations for the results in this study. The slight increase in the absorption rate after ouabain administration is not understood.

Iodoacetate, a metabolic inhibitor which has been shown to inhibit glucose transport in the small intestine of other species, was injected into the isolated segment in the same manner as the ouabain. Iodoacetate decreased the absorption of glucose in the jejunum by 50 to 66 per cent of the original rate (Figure 8) while affecting the absorption from the ileum only slightly (10 to 15 per cent). Iodoacetate had no effect on the rate of absorption from the cecum.

These results indicate that an active mechanism(s) requiring metabolism is involved to some extent in the absorption of glucose from

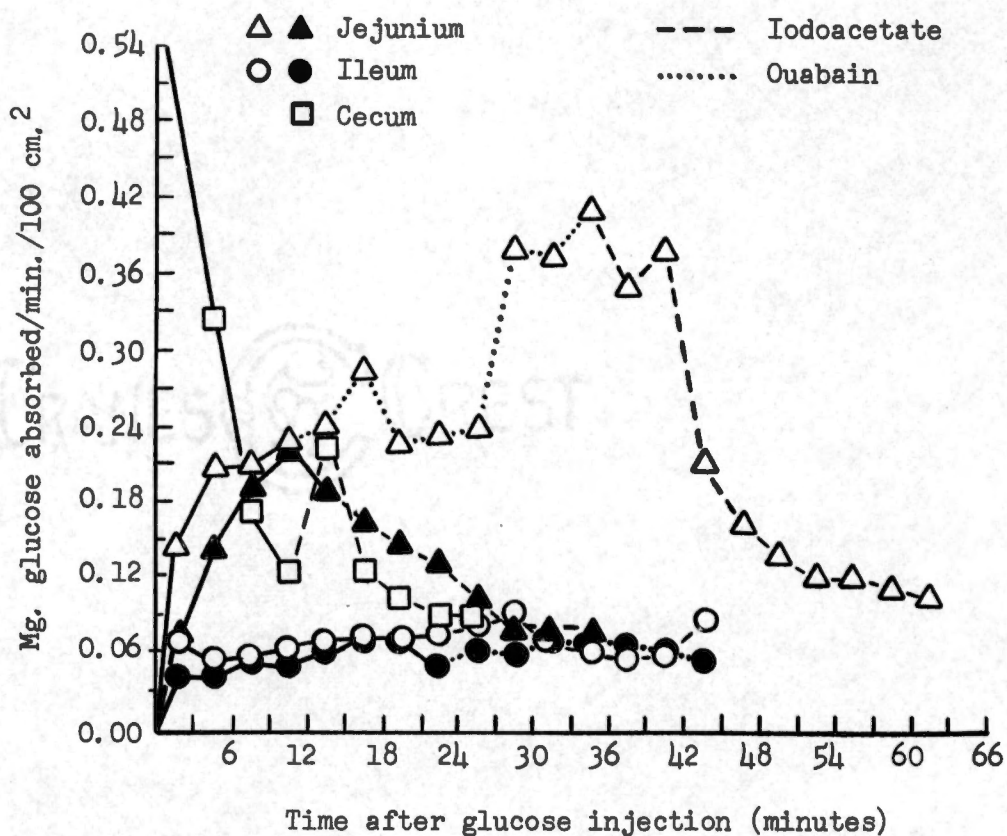


Figure 8. Effect of ouabain and iodoacetate on glucose absorption in the jejunum, ileum, and cecum.

the jejunum and ileum. Possibly the difference in the absorption rates of glucose in the two areas results solely from the difference in the amount of glucose transported actively. A more detailed investigation of this point is necessary for definite conclusions. Since iodoacetate failed to inhibit glucose absorption from the cecum (Figure 8, page 45), an active mechanism, if one is present, is of a different nature than that found in the small intestine. It is possible that the cecum is more permeable to glucose than are the other intestinal tissues and that glucose simply diffuses through the cecal wall more rapidly. Individual data on glucose absorption with the standard deviations are listed in Tables VII through XI of the Appendix.

The rate of glucose absorption from the jejunum of one sheep reached only 0.2 of the rate of other jejunal areas. This observation indicates that in a given section of the small intestine there may be areas where absorption is greatly retarded or that the small intestine of some sheep has very little ability to absorb glucose. A complete biological assay of intestinal samples from these areas as well as from the jejunum and ileum where active absorption rates differ might give some information on the mechanisms involved in active transport.

The values for absorbed glucose obtained by chemical analysis of glucose agreed quite well (within 5 to 8 per cent) with the values obtained by radiochemical analysis. Autoradiograms made from paper chromatography strips used to separate the C-14 labeled materials in the plasma revealed that all of the detectable C-14 was present in the glucose spot. It is possible that a small quantity of C-14 was present

in other compounds such as lactic acid which were in such low concentration that no spots were developed or in volatile compounds such as acetate and propionate which would have evaporated under the nonalkaline conditions employed. These results indicate that the majority of the glucose absorbed in each part of the intestinal tract does appear in the blood as glucose, in agreement with the results of Atkinson, Parsons, and Smyth (1957).

IV. HYDROLYSIS AND UPTAKE OF MALTOSE, STARCH, AND CELLULOSE

The good correlation between the results obtained by chemical and radiochemical analysis justified the use of the chemical analysis for glucose to evaluate the hydrolysis and uptake of maltose and starch. The utilization of the substrates was dependent on the enzyme activity present and the absorption rate of glucose. Complete starch hydrolysis was dependent not only on amylase activity, but also on maltase activity to a great degree. The in vitro results previously presented in this paper indicated that maltase was not a limiting factor in the effectiveness of amylase.

Maltose hydrolysis and uptake was more rapid in the jejunum than the ileum (Figure 9). The in vivo hydrolysis and uptake of maltose in the ileum was about 0.5 times that in the jejunum and corresponds quite closely with the enzyme assay of 62 per cent maltase per gram of mucosal cells in the ileum compared with the jejunum. Increased blood glucose due to the presence of maltose in the small intestine of the older ruminant has been demonstrated by Dollar and Porter (1957), Velu,

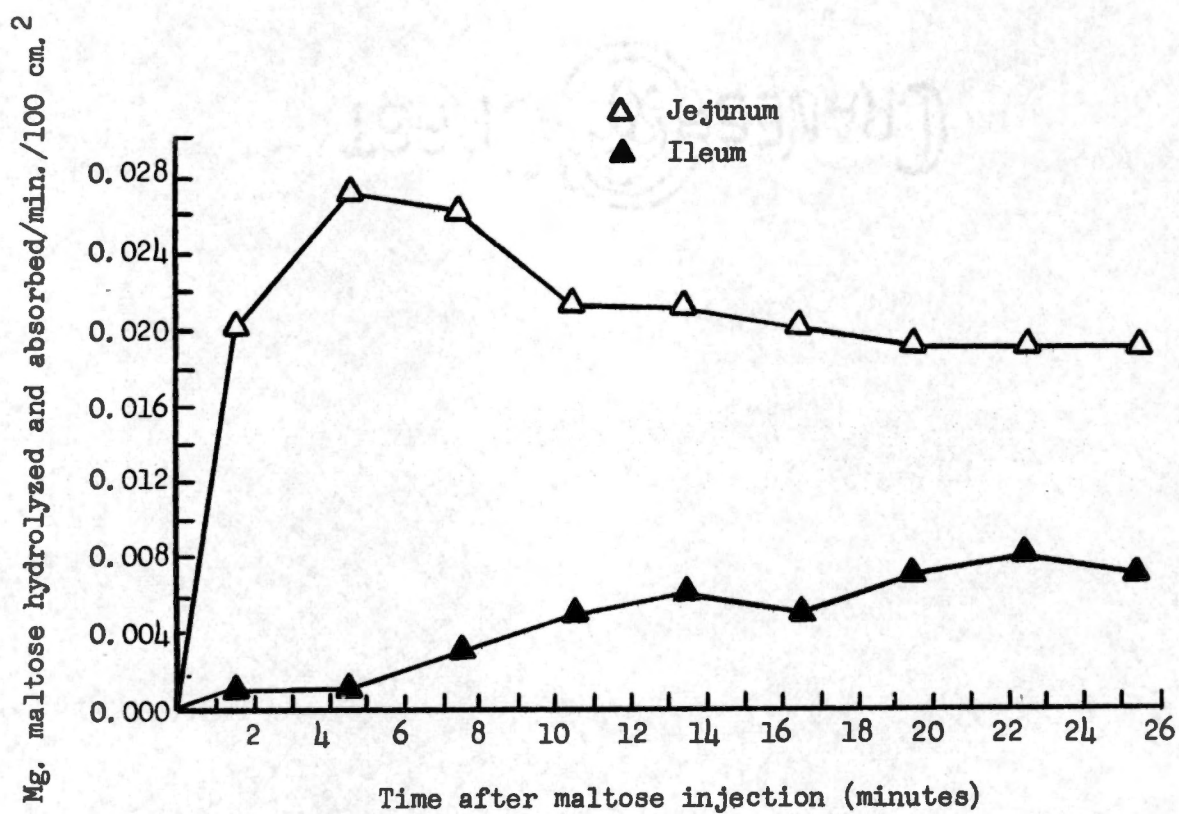


Figure 9. Maltose hydrolysis and uptake in the jejunum and ileum.

Kendall, and Gardner (1960), and Huber et al. (1961); however, no distinction was made between the jejunum and ileum. No hydrolysis and uptake of maltose occurred in the cecum and colon.

During the 30-minute experimental period no starch hydrolysis and uptake from any part of the intestinal tract was observed. Starch hydrolysis and uptake is slow in the ruminant small intestine (Huber et al., 1961) and it is possible that a small amount of hydrolysis and uptake could go undetected. Since the isolated segments were flushed before the solutions were placed in the lumen, no pancreatic amylase was present and any hydrolysis of the starch was dependent on the intestinal amylase. Starch was subjected to pancreatic amylase hydrolysis in the investigation of Huber et al. (1961), and the results of the two experiments could be expected to differ. From the results of the in vitro assays a small amount of starch hydrolysis seems probable. The presence of the starch in the lumen of the intestine for a longer period of time may be required to demonstrate hydrolysis and uptake of the substrate under these experimental conditions.

Placement of C-14 labeled cellulose into the unflushed isolated intestinal segments resulted in C-14 activity in the blood within the first 1.5 minutes from all portions of the intestinal tract. The greatest amount of activity was found in the blood draining the jejunum. A water extraction of the labeled cellulose, which had been stored at 4° C. for 14 months, yielded 12 per cent of the total activity in the water soluble components. Through a subsequent communication with the supplying company it was learned that uniformly labeled C-14 cellulose

stored at 4° C. will break down to water soluble components at the rate of 5 per cent per 6 months time.

An analysis for glucose in the water extract of the cellulose was negative and autoradiograms of the C-14 labeled compounds in the extracts after separation by paper chromatography indicated that there was an array of C-14 labeled compounds present, which was interpreted to mean that appreciable radiolysis had occurred in the cellulose. Radiolysis is minimized by storage below room temperature (0° C. usually adequate). Radiolysis is more likely to occur in compounds stored in the dry state rather than in solution (Wang and Willis, 1965).

Labeled contaminant material in the cellulose made it impossible to evaluate the degree of cellulose utilization. Each cellulose dose, 4.35 mg., was extracted with 3 liters of water to remove all the water soluble impurities. Radiochemical analysis of the last 10 ml. indicated that the soluble contaminants left after the extraction were appreciable and considered too great for further investigation to give reliable results.

The degree of maltose and starch hydrolysis and uptake and the quantity of disaccharidases present in the intestinal tract determined during this investigation indicate that, unless there is a biosynthetic adaptation of carbohydrase production, the mature sheep would be unable to derive enough energy from food stuffs in the intestinal area alone to survive.

CHAPTER V

SUMMARY AND CONCLUSIONS

In vitro assays of the carbohydrase activities in the mucosal cells of the small intestine of mature sheep revealed a higher concentration of all enzymes in the jejunum than in the duodenum and ileum. The maltase level was the highest in all three areas and the amylase and maltase activities were appreciably higher than the activities of lactase, sucrase, and cellobiase.

Glucose absorption from isolated intestinal segments was more rapid from the jejunum than from other areas of the intestinal tract. Active mechanisms appear to play a greater role in glucose transport across the intestinal wall in the jejunum than in the ileum. Two possible reasons for the high rate of glucose absorption in the cecum are that the cecal wall is more permeable to glucose than are the walls of the rest of the intestinal tract, or that there is an active mechanism of transport which is different from those found in the jejunum and ileum. Glucose absorption from the colon was low.

C-14 labeled glucose was the only labeled compound detected in the blood as a result of glucose absorption, but other labeled compounds may have been present in small quantities.

Maltose hydrolysis and uptake in the jejunum was about twice that in the ileum and no utilization occurred in the cecum and colon. The relationship between maltase activity in the mucosal cells of the

jejunum and ileum and the in vivo hydrolysis of maltose in these respective areas was directionally the same and similar in magnitude.

No starch hydrolysis and uptake in any area of the intestinal tract was observed during the 30-minute experimental period. Radio-impurities made it impossible to evaluate the degree of cellulose hydrolysis.

The study points out the need for further investigations on the mechanisms of glucose absorption and the adaptability of digestive enzyme biosynthesis.



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APPENDIX

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TABLE IV
 ENZYME ACTIVITY IN THE MUCOSA OF THE DUODENUM,
 JEJUNUM, AND ILEUM OF MATURE SHEEP

Intestinal segment	Maltase	Amylase*	Lactase	Sucrase	Cellobiase
Enzyme units** x 10					
Duodenum	<u>2.15^b</u>	<u>0.65^b</u>	1.10 ^{a,b}	<u>0.25</u>	0.20
Jejunum	<u>8.05^a</u>	<u>3.23^a</u>	2.07 ^a	0.95	<u>0.45</u>
Ileum	<u>5.00^{a,b}</u>	<u>1.43^{a,b}</u>	0.70 ^b	0.55	<u>0.25</u>
Probability	P<.05	P<.05	P<.05	N. S.	N. S.

*Amylase expressed on a maltase activity basis.

**Enzyme unit = 1 μ mole substrate hydrolyzed/min./gm. mucosal cells (wet weight).

^{a,b}All means in column with same superscript not significantly different.

NOTE: All means underlined not significantly different P<.05.

TABLE V

RATE OF PLASMA FLOW FROM THE JEJUNUM AND ILEUM

Time (min.) after glucose injection	Ml. plasma per 100 cm. ² per min.									
	Jejunum					Ileum				
	Animal no.					Animal no.				
	4	26	6	5	Mean S. D.	1	2	3	4	Mean S. D.
1.5	2.11	2.27	2.00	2.04	2.10 0.13	2.32	1.30	0.93	2.01	1.64 0.29
4.5	2.11	2.16	1.78	2.15	2.05 0.18	2.07	1.30	0.93	2.09	1.60 0.58
7.5	1.69	1.97	1.45	1.80	1.73 0.21	1.80	1.30	0.93	1.93	1.49 0.46
10.5	1.26	1.97	1.18	2.22	1.66 0.16	2.07	1.30	1.09	2.21	1.67 0.55
13.5	0.88	1.82	1.07	2.39	1.54 0.39	1.95	1.17	1.43	2.41	1.74 0.55
16.5	0.61	1.82	1.11	1.63	1.29 0.54	2.12	1.30	1.59	2.41	1.86 0.50
19.5	0.50	1.75	1.18	1.28	1.18 0.51	2.07	1.30	1.65	2.82	1.96 0.65
22.5	0.61	1.75	1.29	1.00	1.16 0.47	2.32	1.30	1.18	2.21	1.75 0.60
25.5	0.69	1.75	1.22	0.59	1.06 0.53	2.20	1.37	1.03	1.61	1.55 0.49

TABLE VI

RATE OF PLASMA FLOW FROM THE CECUM AND COLON

Time (min.) after glucose injection	Ml. plasma per 100 cm. ² per min.									
	Cecum					Colon				
	Animal no.		Mean	S. D.		Animal no.		Mean	S. D.	
	7	8				10	11			
1.5	4.10	7.40	5.50	1.70		8.80	9.30	9.05	0.35	
4.5	3.80	7.40	5.33	1.85		9.10	8.50	8.80	0.42	
7.5	3.70	6.60	4.87	1.52		9.60	8.00	8.80	1.13	
10.5	3.50	5.90	4.57	1.22		8.50	7.50	8.00	0.71	
13.5	3.30	5.10	4.03	0.94		9.10	8.00	8.55	0.77	
16.5	3.30	4.30	3.63	0.57		9.10	8.50	8.80	0.42	
19.5	3.10	4.30	3.50	0.69		8.50	6.60	7.55	1.34	
22.5	2.80	2.80	3.03	0.40		9.40	7.10	8.25	1.57	
25.5	2.50	4.30	3.87	1.20		6.80	5.80	6.30	0.71	

TABLE VII

ABSORPTION OF GLUCOSE FROM THE JEJUNUM OF MATURE SHEEP

Time (min.) after glucose injection	Mg. glucose absorbed per ml. per 100 cm. ² x 10 ⁻²			Mg. glucose absorbed per 100 cm. ² per min.							
	Animal no.			Animal no.							
	1	2	3	Mean	S. D.		1	2	3	Mean	S. D.
1.5	1.048	1.048	0.562	0.886	0.280		0.093	0.063	0.030	0.062	0.031
4.5	2.646	2.430	1.447	2.174	0.630		0.213	0.143	0.080	0.146	0.067
7.5	3.164	3.013	1.901	2.694	0.680		0.207	0.160	0.083	0.150	0.063
10.5	3.856	3.586	2.311	3.251	0.820		0.203	0.190	0.077	0.157	0.067
13.5	4.385	3.910	2.333	3.543	1.010		0.210	0.193	0.053	0.152	0.087
16.5	4.406	3.726	2.300	3.477	1.101		0.220	0.187	0.033	0.148	0.097
19.5	3.964	3.607	-----	3.786	0.180		0.200	0.120	-----	0.160	0.110
22.5	4.007	3.391	1.318	2.905	1.400		0.230	0.160	0.020	0.136	0.107
25.5	3.262	3.391	1.944	2.886	0.800		0.180	0.160	0.033	0.124	0.081

TABLE VIII

ABSORPTION OF GLUCOSE FROM THE ILEUM OF MATURE SHEEP

Time (min.) after glucose injection	Mg. glucose absorbed per ml. per 100 cm. 2 x 10 ⁻²						Mg. glucose absorbed per 100 cm. 2 per min.					
	Animal no.						Animal no.					
	1	2	3	4	Mean	S. D.	1	2	3	4	Mean	S. D.
1.5	0.238	0.281	0.378	0.443	0.335	0.092	0.022	0.015	0.011	0.023	0.018	0.007
4.5	0.702	0.259	0.853	0.702	0.629	0.256	0.038	0.014	0.026	0.037	0.029	0.012
7.5	1.069	0.238	0.907	0.853	0.767	0.360	0.077	0.012	0.028	0.042	0.040	0.028
10.5	1.166	0.270	0.713	0.853	0.751	0.360	0.097	0.014	0.026	0.047	0.047	0.036
13.5	1.210	0.313	0.475	1.188	0.797	0.469	0.094	0.015	0.022	0.071	0.051	0.037
16.5	1.220	0.356	0.637	1.264	0.869	0.435	0.104	0.019	0.032	0.076	0.058	0.040
19.5	1.328	0.421	0.648	0.950	0.836	0.387	0.111	0.022	0.035	0.067	0.059	0.040
22.5	1.166	0.443	0.680	1.015	0.836	0.316	0.109	0.023	0.026	0.056	0.054	0.038
25.5	1.145	0.540	0.648	1.123	0.864	0.310	0.101	0.030	0.022	0.062	0.054	0.036

TABLE IX

ABSORPTION OF GLUCOSE FROM THE CECUM OF MATURE SHEEP

Time (min.) after glucose injection	Mg. glucose absorbed per ml. per 100 cm. ² x 10 ⁻²				Mg. glucose absorbed per 100 cm. ² per min.			
	Animal no.				Animal no.			
	7	8	9	Mean	S. D.	7	8	9
1.5	4.540	6.540	6.934	6.000	1.280	0.293	0.164	0.539
4.5	4.050	3.020	4.212	3.761	0.640	0.242	0.149	0.321
7.5	3.060	2.160	2.430	2.550	0.938	0.172	0.097	0.171
10.5	2.800	1.840	1.868	2.173	0.539	0.151	0.078	0.133
13.5	2.260	1.860	3.985	2.701	1.120	0.116	0.065	0.239
16.5	2.580	2.200	2.408	2.403	0.189	0.131	0.045	0.129
19.5	2.330	4.230	1.825	2.801	1.260	0.116	0.102	0.092
22.5	2.240	2.940	1.436	2.212	0.748	0.101	0.058	0.081
25.5	1.920	3.150	1.080	2.050	1.039	0.076	0.093	0.081

TABLE X

ABSORPTION OF GLUCOSE FROM THE COLON OF MATURE SHEEP

Time (min.) after glucose injection	Mg. glucose absorbed per mL. per 100 cm. ² x 10 ⁻²			Mg. glucose absorbed per 100 cm. ² per min.		
	Animal no.		S. D.	Animal no.		S. D.
	10	11		10	11	
1.5	0.200	0.151	0.178	0.030	0.018	0.016
4.5	0.367	0.232	0.299	0.095	0.033	0.026
7.5	0.367	0.243	0.306	0.088	0.035	0.027
10.5	0.382	0.108	0.246	0.190	0.032	0.020
13.5	0.382	0.270	0.327	0.080	0.035	0.028
16.5	0.340	0.275	0.308	0.047	0.031	0.027
19.5	0.108	0.108	0.108	0.000	0.009	0.008
22.5	0.286	0.027	0.157	0.183	0.027	0.015
25.5	0.421	0.005	0.213	0.293	0.029	0.015

TABLE XI

EFFECT OF OUABAIN AND IODOACETATE ON GLUCOSE ABSORPTION
IN THE JEJUNUM, ILEUM, AND CECUM

Time (min.) after glucose injection	Mg. glucose absorbed per 100 cm. ² per min.					
	Jejunum			Ileum		Cecum
	Animal no.			Animal no.		Animal no.
	20	21	22	23	24	25
1.5	0.080	0.217	0.080	0.046	0.069	0.539
4.5	0.191	0.317	0.299	0.042	0.056	0.321
7.5	-----	0.256	0.165	0.059	0.057	0.171
10.5	0.299	0.196	0.268	0.062	0.053	0.133
13.5 ^a	0.317	0.158	0.222	0.068	0.064	0.239
16.5 ^{b,c}	0.263	0.080	0.163	0.069	0.073	0.129
19.5	0.314	0.151	0.141	0.072	0.073	0.092
22.5	0.328	0.032	0.234	0.051	0.076	0.081
22.5 ^{d,e}	0.279	0.023	0.180	0.063	0.082	0.081
28.5	0.165	0.008	0.153	0.059	0.100	0.081
31.5	0.268	0.013	0.143	0.072	0.074	
34.5	0.200	0.012	0.139	0.152	0.065	
37.5	0.222			0.068	0.060	
40.5 ^f	0.163			0.068	0.060	
43.5	0.141			0.096	0.055	
46.5	0.234			0.150		
49.5	0.180					
52.5	0.153					
55.5	0.143					
58.5	0.139					
61.5	0.133					

^aAnimal 25 injected with iodoacetate.

^bAnimals 21 and 22 injected with iodoacetate.

^cAnimal 20 injected with ouabain.

^dAnimal 23 injected with ouabain.

^eAnimal 24 injected with iodoacetate.

^fAnimal 20 injected with iodoacetate.

TABLE XII

MALTOSE HYDROLYSIS AND UPTAKE IN THE JEJUNUM AND ILEUM

Time (min.) after maltose injection	Maltose hydrolysis and uptake--mg. per 100 cm. ² per min.												
	Jejunum						Ileum						
	Animal no.			Mean			Animal no.			Mean			
	12	13	14	15	S. D.		16	17	18	19	Mean	S. D.	
1.5	0.018	0.027	0.025	0.008	0.020	0.008	0.002	0.000	0.003	0.000	0.001	0.000	
4.5	0.019	0.035	0.029	0.025	0.027	0.008	0.000	0.000	0.003	0.000	0.001	0.000	
7.5	0.004	0.035	0.021	0.045	0.026	0.018	0.009	0.002	0.003	0.000	0.003	0.001	
10.5	0.011	0.018	0.018	0.038	0.021	0.011	0.012	0.003	0.005	0.000	0.005	0.001	
13.5	0.008	-----	0.014	0.043	0.021	0.019	0.010	0.006	0.009	0.000	0.006	0.005	
16.5	0.013	0.015	0.011	0.040	0.020	0.014	0.010	0.002	0.009	0.000	0.005	0.005	
19.5	0.011	0.017	0.015	0.032	0.019	0.010	0.010	0.006	0.010	0.000	0.007	0.005	
22.5	0.005	0.009	0.021	0.039	0.019	0.016	0.015	0.005	0.013	0.000	0.008	0.005	
25.5	0.002	0.019	0.021	0.035	0.019	0.014	0.010	0.008	0.011	0.000	0.007	0.005	