glycolytic pathway but contained triose phosphate isomerase.

2. A new assay method for studying the phosphoglycerate-kinase reaction in the forward direction was developed. The method has the advantage that it can be used with partially purified enzyme preparations containing triose phosphate isomerase and α-glycerophosphate dehydrogenase. The assay method also facilitated an independent study of inhibitors and metal ions in the enzyme reactions.

3. Phosphoglycerate kinase is activated by Mg^{2+} and Mn^{2+} ions, and by no other metal ions tested. It reacts specifically with adenosine and di- and tri-phosphate, but with no other nucleotides.

4. The study of the effect of various thiol-binding agents on the muscle and yeast phosphoglycerate kinases revealed that the muscle phosphoglycerate kinase is a sulphydryl enzyme whereas the yeast enzyme is not.

5. The Michaelis constants for the various substrates and activators obtained with the muscle enzyme are similar to the values obtained with the yeast enzyme. These values are also in good agreement with the data originally reported by Bücher (1947). The Michaelis constants of the muscle and yeast enzymes for phosphoglyceryl phosphate have been determined independently by an isotopic method.

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Digestion and Absorption of Disaccharides in Man

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Disaccharides and starch are well known to constitute the major part of the carbohydrates present in our diet. The general conception of the digestion of disaccharides, as concluded from current text-books of biochemistry, is that disaccharide hydrolases (glycosidases) are secreted with the 'succus entericus' into the intestinal lumen, where the hydrolysis of disaccharides is believed to occur.

During a recent study on intestinal digestion and absorption in man (Borgström, Dahlqvist, Lundh & Sjövall, 1957), it was observed, however, that the glycosidase activity of the intestinal contents during digestion was too low to account for any

considerable digestion of disaccharides. In spite of this, a disaccharide, lactose, was rapidly absorbed when given in the test meal. These findings suggested that the intestinal glycosidases were present in the cells of the intestinal mucosa, and were in good accordance with the results obtained by some previous authors (Cajori, 1933; Ammon & Henning, 1956).

We have now performed a series of experiments in which sucrose, maltose, lactose or, in a few cases, soluble starch have been given in a test meal to men, together with fat, protein and an unabsorbable reference substance for calculating the dilution of the test meal in the intestine. The hydrolysis and the absorption of the carbohydrates, and the carbohydrase activities of the intestinal contents, have been assayed in samples obtained from different levels of the intestine through a plastic tube.

MATERIALS AND METHODS

Twenty-one experiments were performed on 17 male students, 19–29 years old, weighing 58–78 kg. and having a height of 170–188 cm.

Intubation technique

The technique for intubation was essentially that of Blankenhorn, Hirsch & Ahrens (1955), as was used by Borgström *et al.* (1957). The tube was marked so that the distance from the nose to the place in the intestine from which the sample was obtained could be measured. The anatomical localization corresponding to this distance has been carefully investigated by Blankenhorn *et al.* (1955). In the present paper the distance from the nose to the pylorus is assumed to be 60–65 cm.; to the ligament of Treitz (which marks the limit between the duodenum and the jejunum) 85–90 cm.; to the ileocoecal valve 300 cm. In some experiments the balloon containing mercury was replaced by a small steel cylinder, 5 mm. × 14 mm., with hemispherical ends. Intubation was performed in the afternoon of the day before the experiment. In a few experiments the tube was left for 2 days, in order to reach the lower levels of the small intestine. After the experiment the tube was removed by pulling it out through the nose.

Before the test meal was given, the subjects fasted for 12 hr., but were allowed water.

Composition of the test meal

The test meal was of liquid, containing carbohydrate (sucrose, maltose, lactose or soluble starch), fat (either lard or a vegetable oil containing chiefly unsaturated triglycerides), protein (dried egg white), polyethylene glycol (mol.wt. 4000) and egg yolk. The composition by weight of the meal given to each person is: dried egg white, 21·1 g. (contains 18·8 g. of protein), carbohydrate (expressed as the amount of monosaccharides which would be obtained on complete hydrolysis), 56·3 g., fat, 22·3 g., one egg yolk (weighing about 18 g., and containing 5 g. of fat and 3 g. of protein), polyethylene glycol, 4·0 g. and tap water, 295 g. The meal thus had a total weight of about 400 g. and contained 550 kcal.

The carbohydrates used were of analytical-grade purity; sucrose and lactose monohydrate were from Baker Co. (U.S.A.), maltose monohydrate from Pfanstiehl Chemical Co. (U.S.A.) and soluble starch ('amylum soluble nach Zulkowsky') was from Merck A.-G. (Germany). Polyethylene glycol was obtained commercially.

For preparation of the formula the carbohydrate, protein and polyethylene glycol were dissolved individually in water. The solutions then were mixed with the egg yolk in an Ultra-Turrax homogenizer, the fat being slowly added to the solution during homogenization to obtain a smooth emulsion.

The egg yolk was used to obtain a more stable emulsion. Polyethylene glycol was used as a reference substance for calculating the degree of dilution of the test meal in the samples obtained from the intestine, and thus allowing calculation of the degree of absorption of the carbohydrate. Polyethylene glycol passes through the intestinal canal without being absorbed (Borgström *et al.* 1957); it was determined turbidimetrically (Hydén, 1955).

The weight of each carbohydrate in the present paper is expressed as the weight of the monosaccharides which would be obtained on complete hydrolysis, i.e. 1·00 g. of carbohydrate means 0·95 g. of anhydrous disaccharide or 0·90 g. of starch.

Sampling

After the test meal had been ingested, samples were collected for the next 3–4 hr. by siphoning into flasks surrounded by crushed ice. Samples were collected during periods of 30 or 60 min.

With the low glycosidase activity present in the intestinal contents this precaution was sufficient to prevent additional hydrolysis of disaccharides, but possibly not of starch (see below). Analysis for enzymic activity and carbohydrate contents was, generally, performed during the same day as the samples had been collected. Otherwise the samples were stored at −16°, at which they are stable for at least some weeks.

Assay of enzymic activities

Invertase and lactase activities were assayed as described by Dahlqvist (1960), and maltase activity as described by Dahlqvist (1961*a*). One unit of glycosidase activity causes 5% hydrolysis (i.e. the formation of 1mg. of hexose) in 2 ml. of reaction mixture during 60 min. at 37° at optimum pH in 28 mM substrate. The intestinal contents, usually diluted 1:5 or 1:10, were used for the glycosidase-activity determinations without any previous purification. Since the sample thus contained some disaccharide, only the glycosidase activity corresponding to the disaccharide administered was assayed in each case.

Amylase activity was determined as described by Dahlqvist (1961*b*). One unit of amylase activity causes an increase in reducing power corresponding to 1 mg. of maltose monohydrate in 60 min. at 37°, and is thus comparable with the unit for glycosidase activity. This is not the unit for amylase activity used by Borgström *et al.* (1957) with incubation for 3 min. at 25°.

Assay of carbohydrate

Assay of carbohydrate was performed by different methods depending on the kind of carbohydrate present in the test meal. In all cases the amount of free hexose and

disaccharide were assayed *per se*, and in the experiments with starch the average degree of polymerization of the oligosaccharides present was determined in each sample.

Protein precipitation. To 0·5 ml. of the sample was added 1·5 ml. of water, 1·0 ml. of 5% (w/v) $ZnSO_4,7H_2O$ and 1·0 ml. of 0·3 N-$Ba(OH)_2$ (Somogyi, 1945). After mixing and centrifuging, the protein-free supernatant was used for the determination of hexoses and disaccharides present. It could not, however, be used for the determination of starch, a major part of which was precipitated by these reagents. The protein-free supernatant was assumed to contain 0·125 ml. of the original sample/ml.

Assay of sucrose and its hydrolysis products. A suitable amount of the protein-free supernatant (0·5–1·0 ml.) was diluted with water to 2·0 ml. in a test tube. After the addition of 2·0 ml. of Sumner's (1924) 3:5-dinitrosalicylate reagent (most readily prepared as described by Hostettler, Borel & Deuel, 1951) the tube was immersed in a boiling-water bath for 10 min. and then chilled for 2 min. in running tap water. Then 20 ml. of water was added, the contents of the tube were mixed and the extinction at 530 mμ was measured in a spectrophotometer against a blank without sugar, with 1 cm. cuvettes. The amount of glucose and fructose present was calculated from a calibration curve obtained with known amounts (0·2–3·0 mg.) of glucose. Glucose and fructose have the same extinction coefficient with this reagent, but sucrose does not produce any colour. The amount of sugar measured by this method is thus the amount of free glucose and fructose present in the sample. The colour is stable for at least 10 hr.

For measuring the amount of sucrose present, 0·1–0·5 ml. of the protein-free supernatant was diluted with water to 1·0 ml., 1·0 ml. of buffered yeast invertase solution (10 mg. of invertase, analytical, Nutritional Biochemicals Corp., U.S.A., dissolved in 50 ml. of 0·1 M-sodium acetate adjusted to pH 4·5 with 0·1 M-acetic acid) was added and the tube immersed in a water bath at 37° for 1 hr. (The invertase activity was sufficient to hydrolyse 3 mg. of sucrose in less than 10 min.) Then 2·0 ml. of the 3:5-dinitrosalicylate reagent was added and the amount of reducing sugar present measured as described above. The amount of sucrose present in the sample was calculated from a standard series, prepared from glucose, after subtraction of the colour produced by the amount of free sugar originally present.

Assay of maltose and its hydrolysis product. The amount of free glucose in the presence of maltose was measured by the tris–glucose oxidase reagent described by Dahlqvist (1961*a*). A suitable amount of the protein-free supernatant (0·1–0·2 ml.), containing less than 2 mg. of maltose determined as described below, was diluted with water to 0·5 ml. After the addition of 3·0 ml. of the tris–glucose oxidase reagent (Dahlqvist, 1961*a*) the tube was immersed in a water bath at 37° for 1 hr. for development of the colour. The colour produced then was measured in a spectrophotometer at 420 mμ, and the amount of glucose present was calculated from a calibration curve, obtained with known amounts (0·01–0·05 mg.) of glucose.

To measure maltose the reducing power of 0·1–0·5 ml. of protein-free supernatant was determined with the 3:5-dinitrosalicylate reagent. A correction for the free glucose present was made and the amount of maltose calculated from a standard curve, obtained with 0·2–4·0 mg. of maltose.

Assay of lactose and its hydrolysis products. For assay of glucose and galactose in the presence of lactose, 0·1–0·2 ml. of the protein-free supernatant (containing less than 2 mg. of lactose as determined below) was diluted with water to 2·0 ml. in a Folin sugar tube, and 2·0 ml. of acid copper reagent (Tauber & Kleiner, 1932) was added. The tube was heated in a boiling-water bath for 10 min., and then chilled with running tap water for 2 min. Thereafter 2·0 ml. of the arsenomolybdate reagent of Nelson (1944) was added, the contents of the tube were mixed, diluted with water to the 25 ml. mark and mixed again. After the mixture had been standing at room temperature for 15 min. for the colour to be fully developed, reading was made in a spectrophotometer at 660 mμ. Glucose and galactose gave similar standard curves with this method. The standard curve was usually prepared from an equimolar mixture of glucose and galactose (0·1–0·5 mg.). Lactose in amounts less than 2 mg. did not produce any colour.

To determine lactose the reducing power of 0·1–0·2 ml. of protein-free supernatant was determined with the 3:5-dinitrosalicylate reagent. After correction for the colour produced by the glucose and galactose present (glucose and galactose have the same extinction coefficient with this method) the amount of lactose was calculated from a standard curve prepared with known amounts (0·2–4·0 mg.) of lactose.

Assay of starch and its hydrolysis products. These carbohydrates were determined with anthrone by a slight modification of the method described by Scott & Melvin (1953); 2·0 ml. of the sample to be assayed, diluted 1:1000 or more, was transferred to a test tube, which was chilled with tap water. With the tube still in the tap-water bath, 4·0 ml. of the anthrone reagent (100 mg. of anthrone freshly dissolved in 50 ml. of conc. sulphuric acid) was allowed to flow along the wall of the tube to its bottom, and then the contents of the tube were mixed slowly to avoid excessive heating. The tube was covered with an inverted 5 ml. beaker, immersed in a boiling-water bath for 7·5 min. and chilled with tap water for 15 min. The intensity of the colour produced was measured in a spectrophotometer at 625 mμ, with 1 cm. cuvettes. The amount of carbohydrate present was calculated from a standard series prepared from glucose (0·05–0·20 mg.).

Since protein-precipitation reagents also precipitated the major part of starch present in a solution, the anthrone determination had to be performed without protein precipitation. The sensitivity of the method, however, allowed so great dilution of the sample that the proteins present did not interfere. The solutions used for spectrophotometric readings were always perfectly clear. The secretions present in the intestine contained only negligible amounts of anthrone-positive material, that in the intestinal contents from a fasting subject corresponding to less than 1 mg. of glucose/ml. Determination of total carbohydrate with the anthrone reagent in a sample of intestinal contents containing sucrose gave a value in good accordance with that obtained by the method for assay of sucrose described above. Polyethylene glycol does not react with the anthrone reagent.

For determination of the amount of free glucose the tris–glucose oxidase reagent was used on deproteinized samples as described above.

For determination of the average degree of polymerization of the carbohydrate present in the experiments when

the test meal contained soluble starch, the reducing power was measured with the 3:5-dinitrosalicylate reagent. This was performed as described above, but 0·1–0·2 ml. of the sample was used without previous protein precipitation. This did not interfere with the production of the colour, although the solution in most cases became turbid. Before measurement of the intensity of the colour the solution was therefore shaken with 0·1–0·2 g. of Celite and then filtered. The filtrate was perfectly clear. In control experiments with pure sugar solutions, no colour was adsorbed on the Celite.

After correction of the colour measured by that caused by the glucose present, the reducing power was calculated as milligrams of 'maltose monohydrate' present, with a standard curve prepared with that sugar. The intensity of the colour is approximately proportional to the number of reducing groups present for maltose and greater molecules, including polysaccharides (Meyer, Noelting & Bernfeld, 1948; Meyer, van der Wyk & Feng, 1954), and thus a comparison with the total amount of carbohydrate present, as measured with the anthrone reagent, enabled the estimation of the average degree of polymerization of the polysaccharide, oligosaccharide and disaccharide present.

All determinations of mono-, di- or poly-saccharides on fasting intestinal contents, despite their richness in protein, have given values approaching zero. Glycerides and free fatty acids do not react with these reagents. Further, for each substance a characteristic site of absorption was found, beyond which the levels measured were essentially zero.

The accuracy of these methods was supported by the presence of 100% of the ingested disaccharide in the unabsorbed state in samples from the duodenum.

Calculation of the degree of absorption of carbohydrates

In each experiment both the meal administered and the samples of intestinal contents obtained were analysed for polyethylene glycol and carbohydrate. The degree of absorption of sugar in each intestinal sample was calculated by the following formula:

$$\text{Absorption (\%)} = 100\left(1-\frac{S_i.P_m}{P_i.S_m}\right).$$

Absorption (%) means the amount of sugar absorbed, expressed as percentage of the original amount contained in the fraction of the meal which is present in the sample of intestinal contents collected. S_i, concentration of sugar (sum of monosaccharide and disaccharide) in the sample of intestinal contents; P_i, concentration of polyethylene glycol in this sample; S_m, concentration of sugar in the meal administered; P_m, concentration of polyethylene glycol in the meal.

RESULTS

Recovery of the test meal from the intestine. The amount of intestinal contents obtained through the tubing varied between 50 and 400 ml./hr. Sampling was continued for 4 hr. after the ingestion of the test meal, and the amount of polyethylene glycol obtained through the tube during this time varied in individual experiments between 10 and 40% of the total amount ingested, but in most cases was between 20 and 25%. There was no correlation between the amount of polyethylene glycol recovered and the site of collection.

Rate of emptying of the stomach. Of the total amount of polyethylene glycol recovered in samples obtained from the duodenum, about 50% was obtained during the first hour of sampling, 40% during the second hour, 5–10% during the third hour and usually only a few per cent during the fourth hour. The passage of the test meal from the stomach to the duodenum thus seems to be essentially complete in about 3 hr. This is somewhat more rapid than in earlier experiments with a different test meal containing glucose, skim-milk powder and corn oil (Borgström *et al.* 1957). The rate of emptying of the stomach seemed not to be influenced by the particular carbohydrate present in the meal.

Dilution of the test meal in the intestine. The dilution of the test meal in the intestine, as calculated from the concentration of polyethylene glycol in the samples obtained, was two to five times in the upper part of the intestine. At lower levels of the intestine, the test meal was concentrated. These observations are in good accordance with the results obtained earlier (Borgström *et al.* 1957).

Rate of passage through the small intestine. When sampling was made at low levels in the intestine (200–220 cm. from the nose, i.e. the proximal part of the ileum), the distribution of the total amount of polyethylene glycol recovered between the different hours of sampling was more variable. Generally the amount of polyethylene glycol recovered seemed, however, to decrease during the third and fourth hours. Although the time taken for the whole meal to pass through the small intestine cannot be directly measured in these experiments, it can be concluded that after 4 hr. the major part of the meal has reached the ileum.

Absorption of sucrose. Sucrose absorption began in the lower jejunum and probably occurred chiefly in the ileum. No duodenal absorption was found (Fig. 1). The tube did not reach the ileum in the experiments where sucrose was fed. Nevertheless, the observation that more than 50% of ingested sucrose was not absorbed in passing through the jejunum, when added to the known excellent intestinal absorption of sucrose, is strong evidence for the major part of sucrose absorption occurring in the ileum.

The invertase activity of the contents of the jejunum, where sucrose absorption began, was low, usually below 1 unit/ml. (Fig. 2). During the first 2 hr. of absorption, these samples contained 10–70 mg. of sucrose/ml. (Fig. 2), which means that the invertase present could hydrolyse less than 5% of the sucrose/hr. In accordance with this the degree

of hydrolysis of sucrose in all samples was low, never exceeding 10 % (Fig. 3).

The fructofuranosyl group of sucrose is known to be extremely acid-labile, but the low degree of hydrolysis of sucrose in duodenal content, plus the calculated lack of absorption in these samples, indicated that no significant gastric hydrolysis had taken place. In several samples obtained from the stomach, despite the content having pH 3–4, as measured with a glass electrode, the hydrolysis of sucrose was less than 0·5 %.

Absorption of maltose. Maltose was not absorbed in the duodenum, but 50–70 % of that fed was absorbed in the jejunum, and the remainder in the proximal part of the ileum (Fig. 1).

The maltase activity of the intestinal contents was low, in most samples between 1 and 5 units/ml (Fig. 2). The concentration of maltose in the intestinal contents during the first 2 hr. of absorption was between 10 and 70 mg./ml. (Fig. 2). In agreement with this the degree of hydrolysis of maltose in the samples obtained from the region of most active absorption was low, at most about 11 %. In the samples obtained from the upper part of the ileum, where the major part of the maltose already had been absorbed (Fig. 1), the remainder had been hydrolysed to about 50 % (Fig. 3). This high degree of hydrolysis was, however, explained by the low maltose concentration and the relatively high maltase activity of these samples (Fig. 2), which is in sharp contrast with the conditions in the jejunum.

Under the assumption that maltose was hydrolysed by maltase present in the intestinal contents before absorption, the complete hydrolysis and

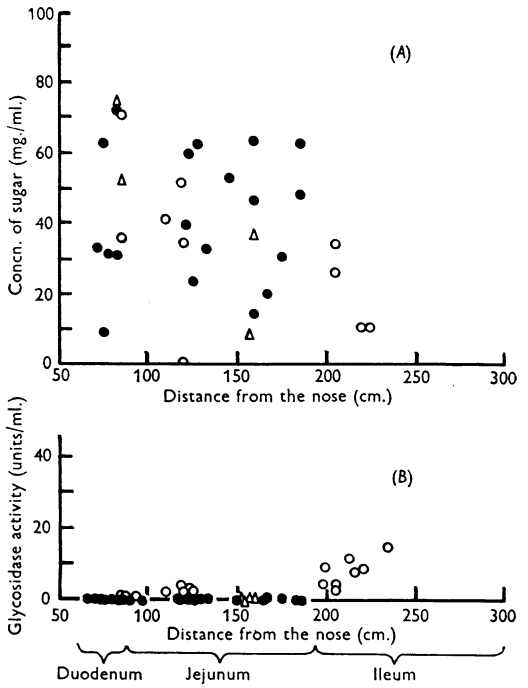

Fig. 2. Concentration of sugar (*A*) and glycosidase activities (*B*) in the intestinal contents during the first 2 hr. after the ingestion of a test meal containing sucrose, maltose or lactose, together with fat and protein. The figures for sugar concentration and enzymic activity may be compared, since 1 unit of glycosidase activity hydrolyses 1 mg. of disaccharide/hr. *A*: ●, Sucrose; ○, maltose; △, lactose. *B*: ●, Invertase; ○, maltase; △, lactase. Zero values for the lactase activity have been obtained also in samples from the duodenum and the proximal part of the jejunum, although these values could not be included in the Figure.

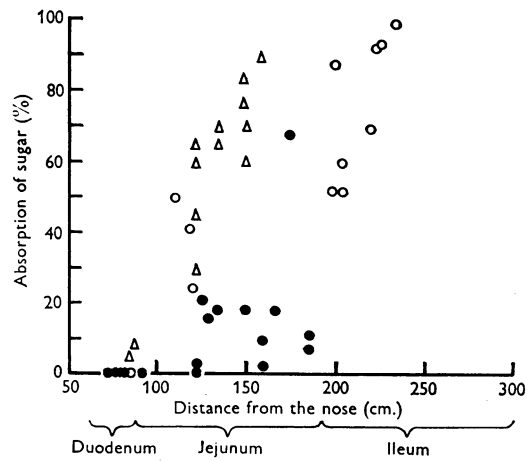

Fig. 1. Degree of absorption of sugar at different levels of the human small intestine after the ingestion of a test meal containing sucrose (●), maltose (○) or lactose (△), together with fat and protein.

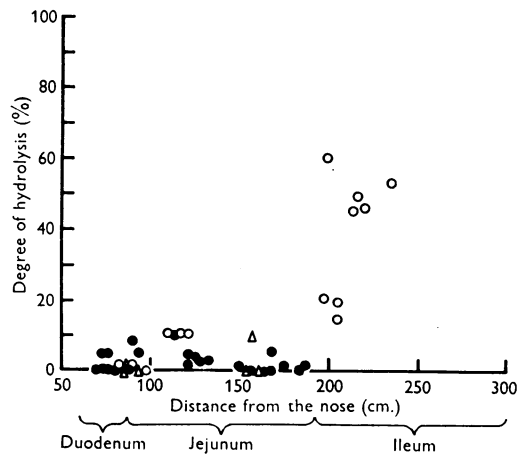

Fig. 3. Degree of hydrolysis of sucrose (●), maltose (○) and lactose (△) present in the intestinal contents at different levels of the human small intestine during absorption.

absorption of maltose would take more than 10 hr. after the sugar had reached the small intestine. It seems, however, that the major part of the maltose present in the test meal was absorbed about 4 hr. after the ingestion of the meal, suggesting adsorption in the disaccharide form. In portions of the test meal which reached the upper part of the ileum during the first hour, 70 % of absorption of maltose had already occurred.

Absorption of lactose. Lactose was absorbed at a higher level in the small intestine than were the other two disaccharides investigated. The absorption of lactose began in the duodenum, and seems to have been completed before the meal reached the lower part of the jejunum (Fig. 1).

In spite of this the lactase activity of the intestinal contents was very weak, being below 0·5 unit/ml. in all samples (Fig. 2). The concentration of lactose in the intestinal contents during the first 2 hr. of absorption was between 8 and 74 mg./ml. (Fig. 2). The degree of hydrolysis of the lactose present was low in all samples (Fig. 3). The complete hydrolysis of the lactose by the lactase present in the intestinal contents would have taken at least 100 hr.; the lactose given in the test meal in fact seems to have been completely absorbed within 3–4 hr.

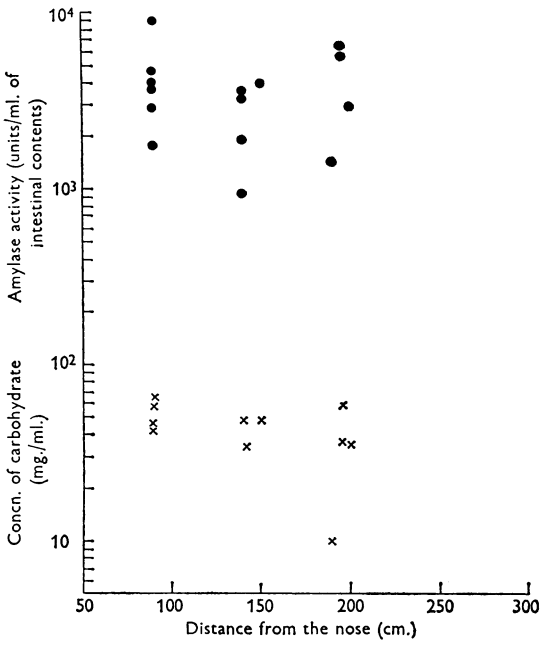

Fig. 4. Concentration of carbohydrate and amylase activity in the intestinal contents during the absorption of a test meal containing soluble starch, fat and protein. Because of the high values for amylase activity the graph has been plotted semilogarithmically. The figures for carbohydrate concentration and amylase activity may be compared, since 1 unit of amylase activity catalyses the formation of 1 mg. of maltose/hr. with starch as the substrate.

Hydrolysis of starch. In three subjects soluble starch was given in the test meal. The concentration of carbohydrate and the amylase activity found in the intestinal contents in these experiments are seen in Fig. 4. This graph has been plotted semilogarithmically, which was necessitated by the high amylase activity of these samples. The concentration of carbohydrate was between 10 and 65 mg./ml., and the amylase activity between 1000 and 9000 units/ml. In accordance with this the degree of hydrolysis was high. The average degree of polymerization of the oligosaccharides present was 2·1–2·4, i.e. at least between 60 and 90 % of the amount of oligosaccharide present was in the form of disaccharide. Between 5 and 10 % of the total amount of carbohydrate present in these samples was free glucose.

Because of the high amylase activity of these samples, the hydrolysis of starch may have proceeded in the flasks after sampling, although these were stored at 0°. On the other hand, since one unit of amylase will hydrolyse starch, forming 1 mg. of maltose/hr. at 37°, the amylase activity of the intestinal contents seems sufficiently high to hydrolyse the starch completely in the intestine within a few minutes. The hydrolysis of starch to disaccharides and oligosaccharides in the intestine thus is an extracellular process, in contrast with the hydrolysis of disaccharides.

DISCUSSION

Both the low glycosidase activity measured and the low degree of hydrolysis of disaccharides in the intestinal contents during the digestion and absorption of the test meal indicate that the hydrolysis of disaccharides in the human intestine does not occur in the intestinal lumen, and that disaccharides are absorbed as such. It is well established that disaccharides infused into the systemic circulation are essentially quantitatively excreted by the kidneys (Verzár & McDougall, 1936). This observation, when added to the high glycosidase activity of mammalian intestinal mucosa (Dahlqvist, 1961*b*), seems to indicate that absorbed disaccharides are hydrolysed by intracellular mucosal enzymes and the monosaccharides pass into the portal system. We have observed (unpublished work) high glycosidase activities in a sample of human intestinal mucosa obtained from the lower part of the ileum. In agreement with the observations on the sites of disaccharide absorption this sample was rich in invertase and maltase activity, but poor in lactase.

Weld (1961) has described particles, named enterolipomicrons, that are present in the secretion

from Thiry loops of the duodenum or of the jejunum in dogs. These particles contain invertase, and Weld is of the opinion that they are the form in which the intestinal glycosidases are secreted. However, no comparison has been made between the amount of disaccharide which the enterolipomicrons are able to hydrolyse per time unit, and the amount of disaccharide actually absorbed by the intestine. On differential centrifuging of homogenates of intestinal mucosa Borgström & Dahlqvist (1958) found glycosidases in the microsome fraction. It seems probable that the enterolipomicrons, which have the same sedimentation properties as the microsomes (Weld, 1961), are formed from disintegrating cells in the intestinal juice.

Results in agreement with our conception of the intestinal glycosidases as intracellular enzymes have been obtained by studies of disaccharide absorption by hamster intestine *in vitro* (Miller & Crane, 1960; Crane, 1960). The experimental details have, however, not yet been published.

Studies *in vivo* and *in vitro* on rats have also indicated that the intestinal dipeptidases are localized inside the cells (Newey & Smyth, 1960). It is thus doubtful whether the 'succus entericus' contains any secreted digestive enzymes at all.

Weidenhagen (1932) suggested that the hydrochloric acid of the stomach was the most important factor in the hydrolysis of sucrose. The low degree of hydrolysis of sucrose in the intestinal contents, and the negligible hydrolysis of this disaccharide in the samples obtained from the stomach in our experiments, demonstrate, however, that the acidity of the stomach contents does not contribute to the hydrolysis of sucrose.

Ugolev (1960*a*, *b*) suggested that the hydrolysis of starch to disaccharides and oligosaccharides in the intestine was effected chiefly by amylase adsorbed to the intestinal wall, considerably higher activity being exerted in this way than when the enzyme is present in free solution. The name 'parietal digestion' was proposed for this mechanism.

In our experiments, however, the amylase activity in the intestinal contents was sufficiently high to hydrolyse the starch in a few minutes. The importance of 'parietal digestion' for the hydrolysis of starch thus could not be confirmed. The hydrolysis of starch to disaccharides and oligosaccharides, in contrast with the hydrolysis of the disaccharides, seems to occur entirely in the intestinal lumen.

Different disaccharides seem to be absorbed in different parts of the small intestine: lactose in the duodenum and the upper part of the jejunum, maltose in the jejunum and the upper part of the ileum, and sucrose in the lower part of the jejunum and in the ileum. The location of the different glycosidases along the human small intestine is not known. The absorption pattern found in these experiments parallels the localization of the different disaccharides over the length of the intestine in the adult pig (Dahlqvist, 1961*b*). The complete absorption of lactose in the proximal part of the intestine (in fact lactose is absorbed at the same level in the small intestine as is free glucose, Borgström *et al.* 1957) seems especially remarkable, since lactose is believed to be utilized with difficulty by adults (Day & Pigman, 1957). The amount of lactose given in the test meal in our experiments corresponds to somewhat more than 1 l. of cow's milk, and seems to be absorbed rapidly, although the total rate of absorption could not be directly measured.

Most of our previous knowledge of the absorption of lactose is, however, based upon experiments with animals, mostly rats (Fischer & Sutton, 1949; Day & Pigman, 1957).

SUMMARY

1. Human subjects have been given the disaccharides sucrose, maltose or lactose, or the polysaccharide starch, in a test meal containing protein, homogenized fat and a non-absorbable reference substance, polyethylene glycol. Intestinal contents were collected by intubation, and the amount of hydrolysis and absorption of carbohydrates were calculated and compared with the carbohydrase activity of the samples.

2. The limited hydrolysis of disaccharides in the intestinal contents, and the extremely low glycosidase activities relative to the calculated amount of carbohydrate absorbed, indicated that the majority of disaccharides were absorbed unhydrolysed, followed by subsequent intracellular hydrolysis.

3. Lactose was absorbed in the duodenum and proximal jejunum, maltose in the jejunum and proximal ileum and sucrose in the distal jejunum and in the ileum.

4. Ingested starch was rapidly hydrolysed to di- and oligo-saccharides by amylase present in the intestinal contents.

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The Precipitation of Polyanions by Long-Chain Aliphatic Ammonium Salts

5. THE INFLUENCE ON PRECIPITATION OF CHANGES IN THE AMINE STRUCTURE*

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Several authors have shown that many polyanions are precipitated from aqueous solution by organic cations containing a long chain of CH_2 units, e.g. cetylpyridinium and cetyltrimethylammonium (for a review see Scott, 1960). Polyanions that take part in this reaction include polyacrylic acid, negatively charged proteins, polyuronides, sulphated polysaccharides, polyphosphates, nucleic acids and borate complexes of neutral polysaccharides. It was concluded (Scott, 1956, 1960) that the reaction is probably general for all water-soluble polyanions.

Scott (1955*a*) demonstrated that the insoluble complexes were salts of the polyanion and the aliphatic ammonium cation. The stoicheiometric combination of, e.g., cetylpyridinium with various polyanions was shown to be of use in the estimation of their acidic groups (Scott, 1955*a*). The complexes are very insoluble (less than 1 mg./100 ml.) and small amounts of polyanion may be precipitated quantitatively from dilute solutions: a useful step in the isolation of polyanions from biological materials, since they can be easily recovered from the complex (Scott, 1960).

The mechanism of the precipitation reaction has been discussed (Scott, 1956) and it was suggested that there were two alternatives, depending on whether the quaternary ammonium ion was considered to react as micellar aggregates or as single ions. The available evidence was insufficient to allow a conclusion to be drawn. The emphasis in this paper is on the influence on the reaction of changes in the structure of the amine. As a result of the investigation it is possible to propose a mechanism of precipitation. Apart from the intrinsic interest of the reaction, it is desirable that it should be better understood so that improvements in the practical application may be made. A preliminary report of some of the results has been given (Scott, 1961).

MATERIALS AND METHODS

Abbreviations. R^+, Organic cation; M^+, inorganic cation; P^{z-}, polyanion with valency z.

Aliphatic ammonium salts

Unbranched primary amines with chain lengths C_6–C_{18} were obtained from L. Light and Co. Ltd. They were converted into the hydrochloride without further purification.

* Part 4: Antonopoulos, Borelius, Gardell, Hamnström & Scott (1961).

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