THE ACTIVE TRANSPORT OF SUGARS BY VARIOUS PREPARATIONS OF HAMSTER INTESTINE*

ROBERT K. CRANE AND PAUL MANDELSTAM**

The Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Mo. (U.S.A.)

(Received April 23rd, 1960)

SUMMARY

The active transport of sugars by the small intestine of the golden hamster has been studied *in vitro* by means of the accumulation of sugar within the tissue against a concentration gradient. Techniques are described for preparing and using three types of intestinal preparation which exhibit characteristics of active transport similar to those of other *in vitro* preparations and of active absorption *in vivo*. One kind, in particular, which consists of small rings of everted intestine was shown to be highly reproducible and, therefore, useful for comparison of the rate and extent of active transport under various conditions. The influence of fasting on active sugar transport was studied and the results are discussed with respect to the source of energy for active transport. The variation in the rate of active transport along the length of the intestine was studied with glucose, galactose and 1,5-anhydro-D-glucitol and found to be similar for all three compounds.

INTRODUCTION

The fact is experimentally well-established that the small intestine possesses an energy-dependent transport process by means of which glucose and certain other sugars can be absorbed against an apparent concentration gradient. This phenomenon has been demonstrated, *in vivo*[1-3], during absorption of sugar from the lumen of intestinal segments into the blood stream and, *in vitro*[4-8], during transfer of sugar from the mucosal to the serosal surface of preparations of intestinal tissue. Since it has also been repeatedly shown that the absorbed or transferred sugar is in a "free" state, there is little doubt that the phenomenon is one of "active transport" as defined by ROSENBERG[9]. However, many characteristics and the mechanism of the process remain largely unknown.

The *in vitro* techniques cited above[4-7] have made it possible to study some properties of the process of active transport such as its response to the cationic environment of the medium[10,11], its specificity with respect to the configuration of

Abbreviations: PPO, diphenyloxazole; POPOP, bis-phenyl oxazolylbenzene.

* A preliminary report of this work was made at the Forty-third Annual Meeting of the American Society of Biological Chemists held at Atlantic City, New Jersey, April 13 to 17, 1959.

** Present address: Department of Pharmacology, Washington University Medical School, St. Louis 10, Mo. (U.S.A.).

sugars presented for absorption[12–15], and its rate with respect to their concentration[7,10,16,17]. However, the significance of much of the collected data suffers from an inherent disadvantage of these techniques. In all of them, the intestine is mounted or prepared in such a way that the medium is separated into two compartments, one bathing the mucosal surface and the other bathing the serosal aspect of the intestinal wall and sugar which is actively transported by the epithelial cells must traverse the entire intestinal wall in order to be measured. Thus, the observed properties of the active transport system include the characteristics not only of the uptake of sugar from the mucosal compartment into the epithelial cells[18] but also of its diffusion through several cell layers as it passes into the serosal compartment of the preparation. Since there is reason to believe that the serosal portion of the intestinal wall is a quantitatively important barrier to diffusion[19], it may be surmised that parameters measured by these methods will have different values than if measured under conditions where this unphysiological diffusion barrier does not exist.

In order to overcome this objection and yet retain the utility of an *in vitro* method, studies have been carried out in which active transport has been measured by means of the accumulation of sugar against an apparent concentration gradient within the tissue itself. With such a method, sugar need enter only the epithelial cells in order to be measured and the underlying tissues serve in these preparations as a repository of transported sugar analogously to the serosal medium in the other *in vitro* methods. In some respects, the experiments are similar to those of KRANE AND CRANE[20] in which it was possible to observe an accumulation of D-galactose against an apparent concentration gradient by slices of rabbit kidney cortex. Previous studies by AGAR, HIRD AND SIDHU[21,22] have shown that surviving strips of intestine can accumulate L-amino acids against an apparent concentration gradient.

MATERIALS AND METHODS

Golden hamsters of either sex and weighing from 150 to 250 g were killed by stunning. The small intestine was removed, flushed with 0.9 % sodium chloride solution, turned inside out[6] and washed in KREBS–HENSELEIT bicarbonate buffer[23].

Tissue preparations

From the washed, everted intestine, four different kinds of preparations were then made as desired. Intact strips: The entire everted intestine was cut with scissors into ring-like pieces having a breadth of 2 to 4 mm. Regions rich in lymphoid tissue (Peyer's patches) were discarded. The pieces were taken for individual flasks in rotation to approximate random sampling and were kept in buffer at room temperature (about 25°) until used. The bicarbonate buffers were maintained in an atmosphere or under a jet of O_2–CO_2 (95:5) at all times, including the time during transfer of tissue or the addition of reagents.

Mucosal and serosal sheets. By means of the technique described by DICKENS AND WEIL-MALHERBE[24], larger segments of intestine were cut apart at about the level of the *muscularis mucosae* into two morphologically distinct sheets of tissue, namely, "mucosal sheets" which were composed, for the most part, of villi held together by the underlying *muscularis mucosae* (see Plate I) and "serosal sheets" which were

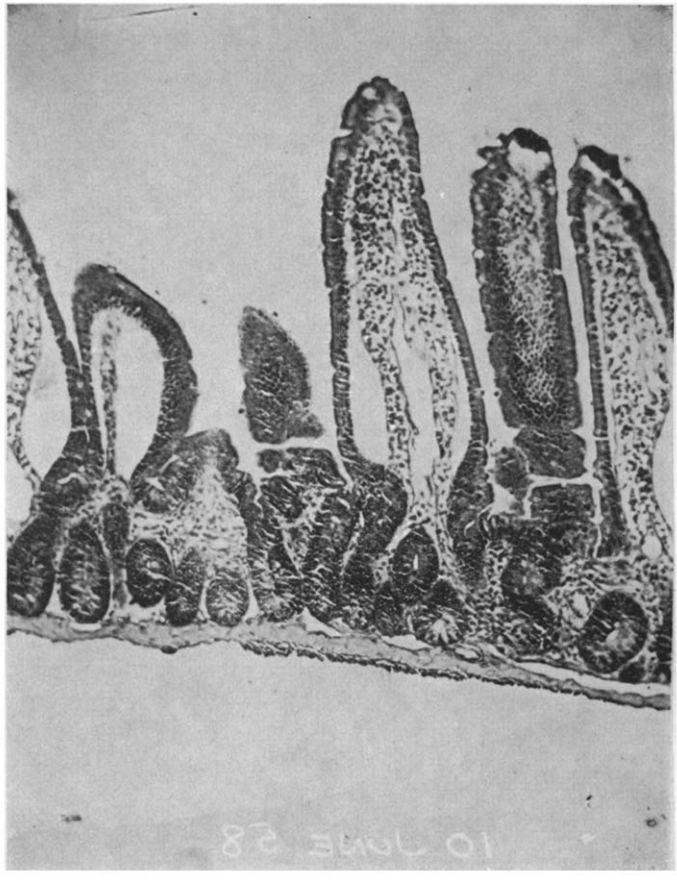

Plate 1. Section of mucosal sheet, fixed in Bouin's fluid and stained with haematoxylin and eosin. Photograph taken under phase contrast illumination, 100 ×.

composed of the submucosal layer, two layers of muscle, the serosa and a few isolated patches of villi (see Plate 2).

Villi: Preparations having no intact structure other than the villi themselves were obtained by fragmentation of mucosal sheets. The sheets were transferred to a 20-ml syringe together with about 10 ml of bicarbonate buffer and forced through needles of successively smaller size. A number 15 needle was used first and was followed by a number 18, then a number 19, and, occasionally when necessary for more complete fragmentation, a number 21. After each treatment, the fragmented tissue was collected by pouring the suspension onto number 16 bolting silk. The tissue retained by the silk was collected by scraping with a spatula and resuspended in buffer. Because the mucous secretions of the intestine unduly lengthened the time required for filtration, the mucosal sheets were incubated for 5 min at 30° in 5 ml of buffer containing 5 mg of a hyaluronidase preparation prior to the fragmentation steps. A bovine testicular hyaluronidase preparation having an activity of 1380 USP units/mg which was a gift of the Wyeth Institute for Medical Research was routinely used, but a less active preparation obtained from Worthington Biochemical Corporation gave similar results

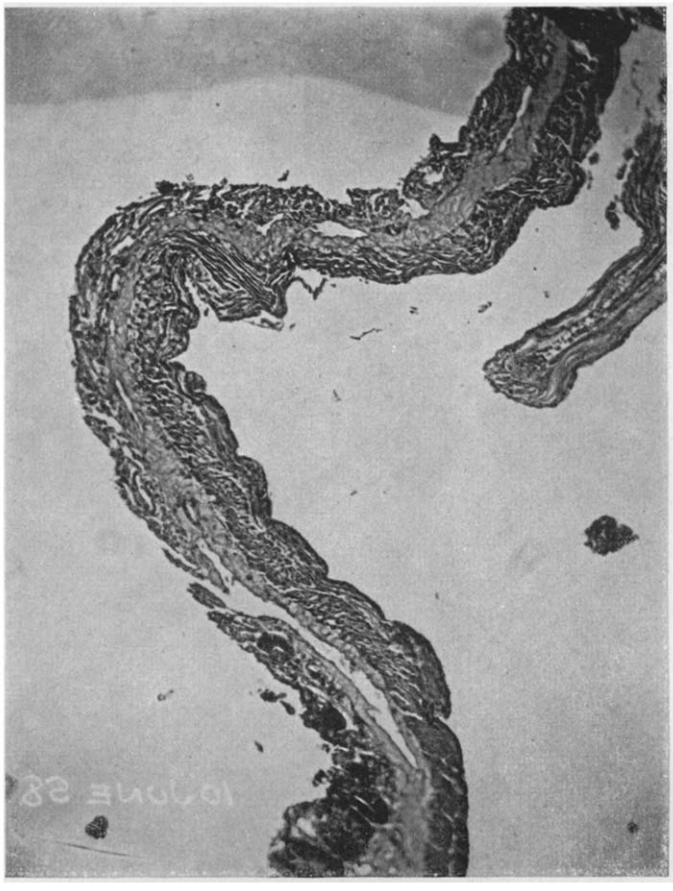

Plate 2. Section of serosal sheet, fixed in Bouin's fluid and stained with haematoxylin and eosin. Photograph taken under phase contrast illumination, 100 ×.

when used in twice the amount. Comparative experiments showed that pretreatment with hyaluronidase never lowered the observed transport activity of the villi preparations and frequently increased it. These preparations of villi, each of which was examined by phase contrast microscopy before use, contained numerous single villi (Plate 3) together with clumps in which from 2 to 6 villi were joined together by one edge of their bases.

Techniques for incubation

Intact strips, mucosal sheets and serosal sheets were incubated in rubberstoppered Erlenmeyer flasks in a shaking incubator of the Dubnoff type manufactured by the Precision Scientific Company. Flasks containing the desired volume of bicarbonate buffer were brought to temperature equilibrium and the tissue was introduced. After an additional 2–3 min period of temperature equilibration, an aliquot of the desired sugar solution was added and incubation was continued for the desired period of time. Incubation was terminated by the removal of the tissue. Except for the mucosal sheets which because of their fragility were weighed before they were introduced into the

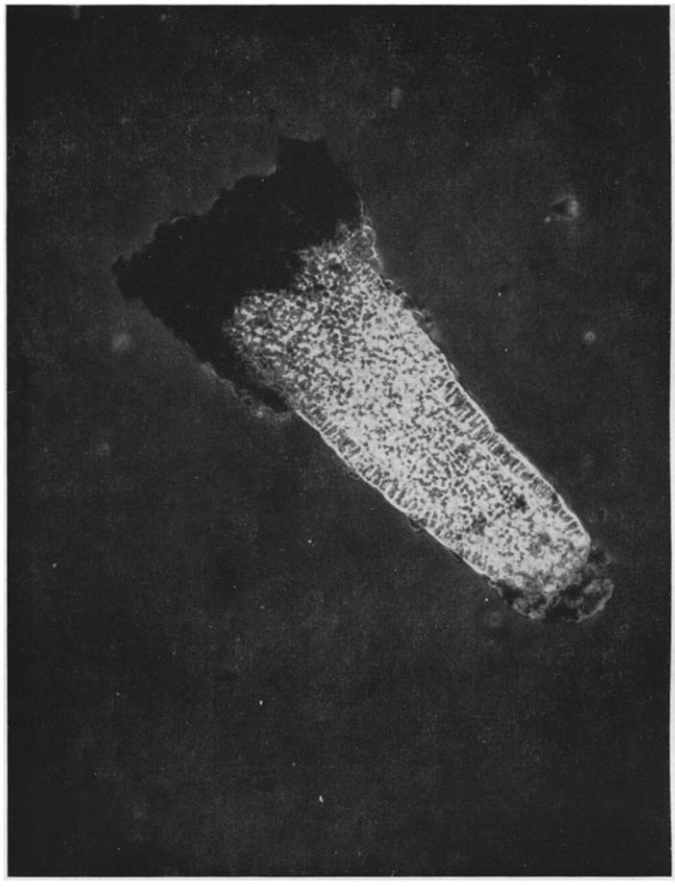

Plate 3. Intact villus. Photograph taken under phase contrast illumination, 100 ×. The dark areas are stained with nigrosin which was added as a test of membrane integrity[54].

flasks for incubation, the recovered tissues were blotted gently on filter paper and weighed on a torsion balance. They were then transferred to 2 ml of 0.19 *M* zinc sulfate solution contained in a conical glass homogenizing tube fitted with a motor-driven ground conical pestle. After homogenization, 2 ml of 0.3 *N* barium hydroxide solution were added and mixed in. The tubes were then centrifuged. In some experiments 4 ml of trichloracetic acid (5 g/100 ml) were used to deproteinize instead of zinc sulfate and barium hydroxide. The protein-free solutions were diluted appropriately with water to bring the concentration of sugar within the range of the applicable method of assay.

Villi preparations were incubated in specially designed glass tubes having a volume of about 8 ml. These tubes were about 12 cm in overall length and were constructed by sealing pyrex tubing of 9 mm, ID, to tubing of 1.5 mm, ID. The tube of narrower diameter was about 2.5 cm in length and was closed at one end with a flattened seal so that it could serve as a cytocrit tube. The volume of a given length of this narrower tube was determined by calibration with mercury. The length of the wider tube was adjusted to fit the International Clinical Centrifuge and the open end was flared slightly to admit a size O rubber stopper. Aliquots, usually 2 ml, of a suspension of villi were

added to the tubes by pipet. The tubes were stoppered and incubated in a vertical position without shaking for 3 min to achieve temperature equilibration. They were then opened to introduce the sugar solution, resealed and incubated in a horizontal position with gentle shaking for the desired period of time. Care was taken throughout to handle the tubes so that the medium and its suspended villi did not enter the capillary portion of the tube prior to centrifugation. Incubation was terminated by centrifugation for 5 min at about 900 $\times$ *g*. The length of the sedimented column of villi was measured, the supernatant fluid was decanted, and the tube, including the portion of the capillary above the packed villi, was dried with absorbent tissue and pipe cleaners. The tube was washed with wetted paper and cleaners and again dried. Then 1.5 ml of zinc sulfate solution were added and the packed villi were suspended by means of a syringe and needle. An equal volume of barium hydroxide solution was then added and mixed in by vigorous strokes of the plunger. Alternatively, in some experiments, 1 to 3 ml of 5 g/100 ml trichloracetic acid were used. Further steps in the preparation for analysis were substantially the same as those described above.

Methods of assay

Glucose was assayed spectrophotometrically by means of the glucose oxidase reaction[25,26] using the glucostat reagent supplied by the Worthington Biochemical Corporation or with the coupled hexokinase, glucose-6-phosphate dehydrogenase reaction[27] using enzymes and triphosphopyridine nucleotide purchased from the Sigma Chemical Company. Galactose was assayed colorimetrically by the SOMOGYI method[28] or, when [1-^{14}C]galactose was used, by virtue of its radioactivity. Non-radioactive 6-deoxy-D-glucose was assayed by the DISCHE AND SHETTLES procedure[29] which is specific for methyl pentoses. The modified ROE method[30] was used for assay of pentoses and the colorimetric procedure described by MCPHERSON[31] was used for histidine.

6-deoxy-D-glucose, 1,5-anhydro-D-glucitol, 1,5-anhydro-D-mannitol, 2-deoxy-D-glucose, and 3-O-methyl-D-glucose were each made radioactive by exposure in the dry state to tritium gas[32] and purified by chromatographic procedures[33]. These compounds, as well as [1-^{14}C]galactose, were assayed by a liquid scintillation technique with the Packard Tri-Carb liquid scintillation spectrometer. To aliquots of deproteinized solutions dried directly in the counting vials, 2 ml of absolute ethanol were added. The vials were then closed and shaken mechanically for 30 min or allowed to stand overnight with occasional shaking by hand. Then 10 ml of toluene containing PRO, 4 g/l, and POPOP, 0.1 g/l, were added and mixed in by swirling. Tests of this procedure in which graded quantities of the non-radioactive species were added together with the tritium-labeled compounds indicated quantitative elution of sugar from the dried residue and satisfactory solubility of the eluted compounds in the scintillation mixture. The observed specific activity of the tritium labeled compounds ranged from 5 to 60 million counts/min/mg. In all experiments, an amount of each radioactive compound was used which would provide for analysis a counting rate at least 10 times background. The non-radioactive analogue was added in the amount necessary to produce the desired final concentration.

Calculation of data

Data are reported as millimolar concentrations of sugar in the tissue and in the

medium or as the ratio of these concentrations, T/M. In order to do this, certain assumptions had to be made in the calculation of tissue concentrations. It was assumed that the sugar was uniformly distributed throughout the tissue water. It would have been preferable to have analysed each tissue layer separately to locate the actively transported sugar as has been done with galactose by McDOUGAL, LITTLE AND CRANE[18]. However, methods of the required sensitivity are not yet available for most sugars. The assumption made, seems to be the one least prejudiced in favor of high tissue to medium concentration ratios. The value of tissue water for intact strips, mucosal sheets and serosal sheets was assumed to be a constant fraction of the wet weight of the blotted tissue. The value was experimentally determined only for intact strips by finding the difference in weight of representative strips before and after drying to constant weight at 100° *in vacuo*. The average value found in the samples used was 82.5 % of the wet weight; the value used in the calculations was 80 %. A correction for the amount of sugar which entered the tissue by routes other than through the active transport process was also made in some experiments. This correction was based on the tissue concentration after 15 min of incubation at 37° of a compound, 1,5-anhydro-D-mannitol, which is not actively transported[12]. This concentration was found to be close to 30 % of the medium concentration. In the strict sense, this correction, although similar in value to extracellular space corrections for other tissues[20,34], is not a correction for extracellular space but for passive diffusion of sugar into the tissue. Calculations of tissue concentrations in intact strips, thus, took the following form:

$$\text{m}M\text{ (tissue)} = \frac{\text{m}M\text{ (filtrate)} \times \text{homogenate volume}}{\text{wet weight of tissue} \times 0.8} - 0.3 \times \text{m}M\text{ (medium)}$$

Since the correction for passive diffusion into mucosal sheets and serosal sheets was not experimentally determined the correction, 0.3 × mM (medium), was omitted in the calculations for these preparations.

Calculations of tissue concentrations for villi take a slightly different form because of the technical differences in the experiments. Recent measurements of the apparent extracellular volume of the packed villi have been made by Dr. I. BIHLER, using [1-^{14}C]mannitol. This compound can be used for this purpose with erythrocytes[34] and the isolated perfused heart[35] and has been shown to be not absorbed from an intestinal loop in the intact rat[37]. The measurements with villi indicate a value in the range of 75 to 80 % for the extracellular volume. A volume of 75 % has been assumed. Since the villi are measured by volume rather than weight, no correction for the space occupied by tissue solids has been made. Calculation of tissue concentrations in villi, thus, took the following form:

$$\text{m}M\text{ (tissue)} = \frac{\text{m}M\text{ (filtrate)} \times \text{homogenate volume} - 0.75 \times \text{villi volume} \times \text{m}M\text{ (medium)}}{0.25 \times \text{villi volume}}$$

EXPERIMENTAL

Studies with intact strips, general

Active transport of sugar by intact strips of intestine was found, like active transport of sugar by other *in vitro* preparations[4-6], to be characterized by (a) dependence on aerobic metabolism, (b) inhibition by phlorizin and (c) specificity with

respect to the sugars that are actively transported. For example, as shown in Table I, 6-deoxy-D-glucose was accumulated within the tissue to a high concentration relative to the medium, T/M = 17.2. Accumulation did not occur when the tissue was deprived of oxygen, when 4,6-dinitro-*o*-cresol, a potent inhibitor of aerobic phosphorylation[38], was added nor in the presence of phlorizin. Moreover, the specificity of active transport by this preparation, insofar as it was examined (see Table II), paralleled the findings with the everted sac preparation[12–15]. All actively transported compounds have, in common, a pyranose ring, a methyl or substituted methyl group at carbon-5 of the ring and a hydroxyl group in the glucose configuration at carbon-2. Compounds which lack one or more of these structural features are not actively transported.

TABLE I

ACTIVE TRANSPORT OF SUGARS BY INTACT STRIPS OF HAMSTER INTESTINE UNDER VARIOUS CONDITIONS

Incubations were at 37° for 40 min. Concentrations of sugars and added components were as follows: 6-deoxy-D-glucose, 0.25 m*M*, 3-O-methyl-D-glucose, 1.0 m*M*, D-xylose, 1.0 m*M*, phlorizin, 1.0 m*M*, and 4,6-dinitro-*o*-cresol, 0.1 m*M*. Nitrogen refers to a gas space of N_2–CO_2 (95:5) instead of the usual oxygen–carbon dioxide mixture. Animals were fasted 16 h. The data for D-xylose are not corrected by the factor of 0.3 × m*M* (medium). T/M is the ratio of the final tissue concentration to the final medium concentration.

Test sugar	*Condition*	*T/M*
6-deoxy-D-glucose	Control	17.2
	Nitrogen	0.7
	Phlorizin	0.7
	4,6-dinitro-*o*-cresol	1.0
3-O-methyl-D-glucose	Control	3.4
	Nitrogen	0.6
D-xylose	Control	0.4
	Nitrogen	0.3

TABLE II

ACTIVE TRANSPORT OF VARIOUS SUGARS AND RELATED COMPOUNDS BY INTACT STRIPS OF HAMSTER INTESTINE

Incubations were at 37° for the time periods indicated. T/M is the ratio of the final tissue concentration to the final medium concentration.

Compound	*Incubation period (min)*	*Concentrations, mM Medium*		*Tissue*	*T/M*
		Initial	*Final*		
Glucose	20	1.0	0.07	14.2	203
	30	4.0	1.46	32.0	21.8
Galactose	20	4.0	1.81	33.0	18.2
3-O-methyl-D-glucose	40	3.8	3.3	7.6	2.3
1,5-anhydro-D-glucitol	40	1.0	0.94	5.5	5.8
6-deoxy-D-glucose	35	0.25	0.084	2.12	25.3
7-deoxy-D-glucoheptose	30	1.0	0.73	12.6	17.3
	30	0.073	0.013	1.06	82
2-deoxy-D-glucose	15	1.2	1.2	0.37	0.31
1,5-anhydro-D-mannitol	15	1.2	1.2	0.30	0.25
D-xylose	40	5.0	4.9	1.5	0.38
L-arabinose	30	1.0	1.0	0.30	0.30

The final relative concentrations between tissue and medium, T/M, shown in Table II are of additional interest in that they provide insight into the concentrative potential of the active transport process. At low external concentrations, glucose, for example, was accumulated to a T/M of over 200 and 7-deoxy-D-glucoheptose to T/M of over 80. In view of the fact that these values are based on average tissue concentrations, it is very likely that the T/M between the epithelial cells and the medium is even higher[18]. Under these conditions, only a small absolute amount of sugar was accumulated. When higher concentrations were used, the value of T/M was lower. The apparent concentration gradients for glucose are especially noteworthy because accumulation of this sugar reflects the extent to which active transport is more rapid than utilization. The differences in observed T/M among the sugars are large and it is likely that one reason for these differences is the differences in the apparent K_m values for the transport process[39]. When sugars with different K_m values are present at the same initial non-saturating concentration, differences in the rate of transport and, consequently, in the extent of accumulation may be expected.

The final relative concentrations of compounds which were not actively transported was in the range 0.3 to 0.4. It would be reasonable to expect that T/M for these compounds would approach or equal 1. Why it did not do so is not clear from the presently available information. One would suppose that sugars could gain entrance to the total extracellular space of the tissue and thus to all of the cells by means of the cut margins of the strips. To explain the apparent restriction of these sugars to a tissue volume which is not much, if any, larger than the extracellular space one may suppose either (a) that the cells of the tissue (muscle cells, epithelial cells, submucosa, etc.) are relatively impermeable to these sugars or (b) that these sugars enter the cells at a rate which is not much greater than the rate at which they are utilized. Recent studies indicate that a somewhat greater volume of distribution can be obtained if incubation is prolonged for a period of 1 h or more.

Reproducibility

A major advantage of the technique with intact strips is its utility for experiments containing multiple variables. With the usual type of *in vitro* preparation in which a long segment of the intestine is used, precise kinetic studies on a routine basis have not been possible owing to variations in the rate of active transport throughout the length of the small intestine[40]; even adjacent segments exhibit significantly different rates. To get around this difficulty, resort has been made to reusing the same segment of intestine during several successive time periods[7] or to using the entire intestine from a large series of animals[5,16,17]. However, kinetic studies are most easily performed if each of several flasks in a single experiment contains tissue of the same or nearly the same potential activity. The present technique, as the results in Table III indicate, provides the required degree of reproducibility to make this possible. As is described in the legend to this table, intact strips from the intestine of one animal were divided among three flasks and incubated with sugar under identical conditions. The amount of accumulated tissue sugar, either glucose or galactose, was then measured. It was found to vary within reasonably small limits. Since both glucose and galactose are utilized by the intestine, the use of these two sugars is believed to represent the most stringent test of the technique.

TABLE III

STUDIES ON THE REPRODUCIBILITY OF THE MEASUREMENT OF ACTIVE TRANSPORT OF SUGARS WITH INTACT STRIPS OF HAMSTER INTESTINE

Each experiment was carried out with the intestine from a single animal divided among three flasks as described in the METHODS section. The temperature of incubation was 37°. The initial concentration of sugar was 5 m*M* in all cases.

Sugar	*Duration of fast (h)*	*Duration of incubation (min)*	*Concentration mM*	
			Final medium	*Tissue*
Glucose	48	10	2.47 ± 0.35	30.0 ± 1.2
	48	15	2.06 ± 0.33	34.8 ± 2.1
Galactose	24	10	2.43 ± 0.19	26.7 ± 1.9
	24	20	2.05 ± 0.25	32.4 ± 2.9
	48	15	2.76 ± 0.09	27.1 ± 0.51
	72	15	2.72 ± 0.24	22.3 ± 0.54
	96	15	2.40 ± 0.10	39.3 ± 1.2

The effect of fasting

Since tissue from non-fasted animals was found to produce significant quantities of free glucose during incubation (for example, 350 mg of tissue from a non-fasted animal produced, in 5 min of incubation, 1 m*M* of glucose in the tissue water and 0.03 m*M* in the 4 ml of medium) and tissue from fasted animals yielded no detectable free glucose, experiments were carried out to see whether fasting would be deleterious to the active transport process. Earlier studies[41,42] in the intact animal indicated that fasting for periods of time similar to those used in the present study greatly reduced intestinal absorption of sugar. Careful studies of the effect of fasting periods of less than 24 h have not been done, so it is possible that during the first day there is a loss in the transport activity of the intestine as measured by the intact strip technique. However, from the 24th to the 96th h of fasting, (see Table III) there was no continued loss with time and there was not more difference than may have been expected as a variation between individual animals. In fact, the greatest accumulation was observed in strips from the animal that had been fasted for 96 h. Some of the experiments in the present report were carried out prior to the studies on the effect of fasting and, as noted in the legends, the animals were fasted for only 16 h. For all other experiments in this and subsequent reports the animals were fasted at least 48 h prior to use.

Location along the intestine of the active transport process

FISHER AND PARSONS[16,17,43], using a different *in vitro* technique[4] and a different species, found different relative rates of active transport for glucose and galactose along the length of the intestine and suggested on that basis that the two sugars may be absorbed by separate processes. On this account, the pattern of variation in the extent of active transport measured by the intact strip technique was studied. For these experiments, the intestine from a single animal was used for each sugar studied. The excised and everted intestine was laid, without stretching, alongside a 30 cm rule and successive 4–5 cm portions, beginning at the pylorus, were taken and separately cut into the usual small pieces. The pieces were incubated in vessels identified as to the portion of the intestine from which they were obtained and were kept in order through-

out the rest of the procedure. Three sugars were used; namely, glucose, galactose and the non-utilizable compound 1,5-anhydro-D-glucitol. The results of these experiments are shown in Fig. 1. In all three experiments, the greatest extent of accumulation was found with tissue taken from the middle portion of intestine.

Rate and extent of accumulation

Accumulation of sugar with the formation of an apparent concentration gradient between the tissue and the medium occurred rapidly at 37°, as is illustrated by the time courses of accumulation of the sugars, glucose and galactose, shown in Fig. 2. The initial rate, however, was not maintained; it fell off with time as an apparent maximal extent of accumulation was attained. Although the maximal value was not measured in the experiments shown in Fig. 2, it was usually found in other experiments to be about 30–40 mM and to be attained in the time period between 15 and 20 min.

Calculations of initial rates from the curves presented in Fig. 2 and comparison of these rates with some values for various metabolic parameters collected from the literature (Table IV) show active sugar transport to be a major activity of intestinal tissue. Glucose transport measured by the strip technique is more than three times faster, on a molar basis, than oxygen consumption, lactate formation or glucose utilization. Galactose transport is 16 times more rapid than galactose utilization. The strip technique shows, moreover, a 2–3 fold higher rate of transport than the reported value for everted sacs. The value of 1080 μmoles/g wet weight/h for galactose transport is taken from a later paper of this series[18] in which analyses of the epithelial cell layer

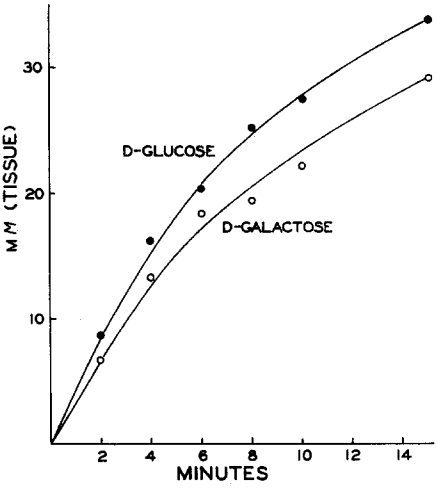

Fig. 1. The distribution of active sugar transport activity along the length of the hamster intestine. The temperature of incubation was 37°. The initial concentrations were, as follows: galactose, 10 mM, glucose, 5 mM, and 1,5-anhydro-D-glucitol, 5 mM. The duration of all incubations was 15 min.

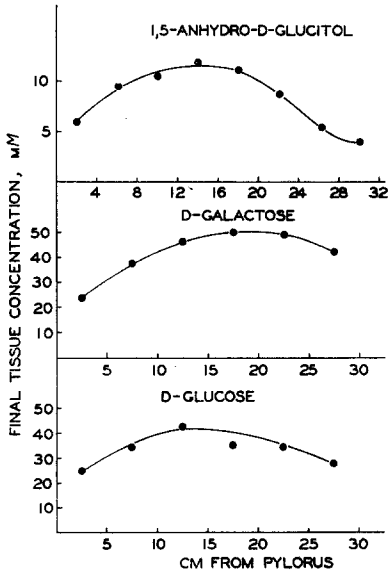

Fig. 2. The accumulation of sugar within intact strips as a function of time. Temperature of incubation was 37°. The initial concentration of sugar was 5 mM in both instances. The rates are not be to compared as intestine from different animals was used for the two sugars.

TABLE IV

RATES OF VARIOUS METABOLIC ACTIVITIES AND OF ACTIVE SUGAR TRANSPORT FOR HAMSTER INTESTINE

Values taken from the work of others are average values of the published data and they have been corrected to a wet weight basis assuming a dry to wet weight ratio of 0.20. Except for the single value marked with an asterisk, which is based on the wet weight of the epithelial cell layer, all weights refer to the entire intestinal wall.

Activity measured	*μmoles/g wet weight/h*	*Reference*
Active transport by the strip technique:		
Glucose active transport	417	See text
Galactose active transport	338	See text
	1080*	See text
Active transport by the everted sac technique:		
Glucose active transport	152	44
Galactose active transport	134	44
Oxygen consumption	123	45
Aerobic lactate formation	59	45
Anaerobic lactate formation	132	45
Aerobic glucose utilization	124	6
Aerobic galactose utilization	20	46

were performed. If this rate were calculated on the basis of the wet weight of the entire intestinal wall, it would be somewhat greater than the other values obtained with the strip technique.

The results of studies of the influence of the initial concentration of sugar in the medium on the apparent final tissue concentration and the final relative concentrations, T/M, are shown in the diagrams in Fig. 3. Both parameters were influenced by

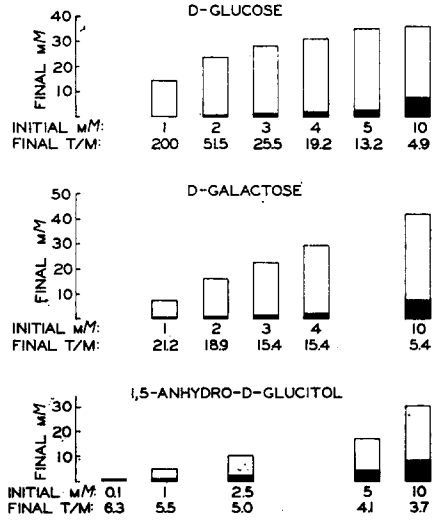

Fig. 3. The active transport of sugars by intact strips as a function of concentration. The total height of the column represents the tissue concentration; the height of the cross-hatched portion, the final concentration in the medium. Incubation temperature was 37°. Duration of incubation was 20 min for glucose and galactose, 15 min for 1,5-anhydro-D-glucitol.

the initial concentration of sugar. In part, this must have been due to the influence of the concentration on the rate of transport inasmuch as the range of concentrations used included the apparent K_m (see ref. 39). However, comparison of the data for all three sugars at the 10 mM concentration level suggests the possibility that there is a limiting concentration which the tissue can accumulate. This aspect will be the subject of future investigations, since studies in this area should provide some insight as to whether the observed concentrations should be viewed as the result of a unidirectional inward active transport of sugar balanced at a steady-state by outward diffusion, or in some other way.

Studies with mucosal sheets, serosal sheets and villi

Mucosal sheets and villi are able to accumulate sugar against an apparent concentration gradient and the accumulation is inhibited by anaerobiosis, or the addition of phlorizin, 4,6-dinitro-*o*-cresol, or a second actively transported sugar (Table V). In these ways, active transport in mucosal sheets and villi is like active transport in intact strips. The mucosal sheets, owing to their fragility, are not well suited to the routine study of active transport, but the villi seem to be. *In vivo*, the villus is the physiological absorption unit; that is, sugar transported by the epithelial cells passes into the "core" of the villus where it enters the blood stream. The villi preparation is a means for studying some of the characteristics of this physiological unit. The initial rate of accumulation using 6-deoxy-D-glucose as the substrate varied widely with villi preparations from different animals but it was usually in the range of 20–80 μmoles/ml intracellular water/h. Oxygen consumption of villi prepared from the intestine of a 48-h fasted animal and incubated without added substrate was about 280 μmoles/g wet weight/h; a value which is comparable to the oxygen consumption value given in Table IV for everted sacs. With villi, as with intact strips, the rate of

TABLE V

ACTIVE TRANSPORT OF 6-DEOXY-D-GLUCOSE BY "MUCOSAL SHEETS" AND VILLI UNDER VARIOUS CONDITIONS

Incubation temperature 37°. Duration of incubation was 40 min with mucosal sheets, and 15 min with villi. Initial concentration of 6-deoxy-D-glucose in all experiments was 0.25 mM. Concentrations of other added compounds were as follows: phlorizin, 1 mM 4,6-dinitro-*o*-cresol, 0.1 mM, galactose, 10 mM, and glucose, 10 mM. Nitrogen refers to a gas space containing N_2–CO_2 (95:5) in place of the usual oxygen–carbon dioxide mixture. Animals were fasted 16 h. The villi for the experiments reported in this table were made without prior treatment with hyaluronidase. T/M is the ratio of the final tissue concentration to the final medium concentration.

	Final relative concentration, T/M	
	Mucosal sheets	*Villi*
Control	6.6	—
+ Nitrogen	1.4	—
Control	12.5	10.2
+ Phlorizin	1.9	1.0
Control	27.2	8.6
+ 4,6-dinitro-*o*-cresol	2.0	2.2
Control	16.2	23.0
+ Galactose	5.6	7.8
Control	11.1	27.0
+ Glucose	2.4	1.4

sugar accumulation fell off rapidly with time and the extent of accumulation was maximal in about 10–15 min. Although one might have expected the villi to be a very "leaky" preparation, inasmuch as sugar which leaves the serosal side of the epithelial cell is returned to the medium rather than trapped in the underlying tissues as it is in the intact strip, the gradients which are established (Table V) are large. This conforms with the finding of McDOUGAL *et al.*[18] that sugar is accumulated within the epithelial cells.

It was found instructive to compare active transport in the intact strip with active transport in its sub-units, the mucosal sheet and serosal sheet. The results of these studies are shown in Table VI. On the one hand, the intact strip was incubated in a medium containing 6-deoxy-D-glucose. After incubation, it was separated as described in the section on MATERIALS AND METHODS into a mucosal sheet and a serosal sheet and the separated portions were analyzed separately. On the other hand, the separation was made prior to incubation and the mucosal sheet and serosal sheet were incubated separately. When separation was made after incubation it was found that both portions contained accumulated sugar and at approximately equal concentrations. However, when separation was made before incubation, only the mucosal sheet contained sugar at a high relative concentration. Obviously, the epithelial cells are required for active transport of sugar. This difference between the preparations, however, does not extend to the transport of amino acids. The data in Table VI show that the amino acid, L-histidine, is accumulated by both mucosal and serosal sheets when incubated separately. It thus appears amino acid can be accumulated within the tissue independently of the epithelial cells.

DISCUSSION

Data presented above demonstrate that accumulation of sugar to high relative tissue concentrations with *in vitro* preparations of hamster small intestine can serve as a highly reproducible system for the study of the characteristics of intestinal active sugar transport. With the availability of this technique, it was possible to make a number of other observations which are relevant to certain aspects of the active transport process.

TABLE VI

THE ROLE OF THE MUCOSAL AND SEROSAL PORTIONS OF THE INTESTINAL WALL IN THE ACCUMULATION OF 6-DEOXY-D-GLUCOSE AND L-HISTIDINE BY INTACT STRIPS OF HAMSTER INTESTINE

ncubation temperature was 37°. The concentration of 6-deoxy-D-glucose was 0.25 m*M* and of -histidine 2.48 mg/ml. The duration of incubation in experiments A and C was 30 min, in Expt. B, 2 min. For further details consult the text. The data for L-histidine are not corrected for the factor .3 m*M* (medium). In Expt. A the mucosal and serosal sheets were prepared from an intact strip fter its incubation. In Expts. B and C, the mucosal and serosal sheets were prepared before incubation and incubated separately.

Expt.	*Compound*	*Relative concentration, T/M*		
		Intact strip	*Mucosal sheet*	*Serosal sheet*
A	6-deoxy-D-glucose	28.6	28.2	30.6
B	6-deoxy-D-glucose	—	15.1	1.4
C	L-histidine	4.8	3.8	2.9

First, there is the question of the source of the energy which is required for accumulation. When glucose and galactose serve as the substrate for active transport they are also utilized and energy generated by their utilization may provide the necessary driving force. However, when non-metabolizable compounds are accumulated, energy is probably derived from another source. In the experiments reported in Fig. 3, the animals had all been fasted for 48 h. No utilizable substrate was added, yet nearly as much 1,5-anhydro-D-glucitol as glucose or galactose was accumulated in an equivalent time period. Oxygen consumption of intact strips and of villi was rapid and did not depend on the addition of substrate. In fact, several unsuccessful attempts were made to influence appreciably the endogenous oxygen consumption of villi by the addition of various sugars, Krebs cycle intermediates, etc. These observations suggest that energy for the active transport of sugars under the present experimental conditions is provided by the oxidation of non-carbohydrate metabolites, possibly fat or fatty acids. It would seem relevant to this point that DICKENS AND WEIL-MALHERBE[24] observed with rat intestinal mucosa a low respiratory quotient which was not greatly influenced by the addition of glucose to the medium.

Second, there is the question of the location of the active transport process in different regions of the small intestine and in other parts of the gastrointestinal tract. Tests of active sugar transport with stomach and proximal colon of the hamster were made by the intact strip technique and compared to the results obtained with intact strips of small intestine prepared from the same animal. Following incubation at 37° in a medium containing 6-deoxy-D-glucose the small intestine was found to have accumulated this compound to a final relative concentration of T/M = 25.4, whereas for the stomach the final relative concentration was T/M = 0.62 and for large intestine, T/M = 0.61. The active process, then, is restricted to the small intestine, though not to as limited a portion of it as appears, for example, to be true for active Ca^{++} transport[47]. The activity is present throughout the small intestine although it varies along its length with the greatest extent of accumulation measured by the intact strip technique observed in the mid-portion (Fig. 1). In previous studies with a different technique[4] in the rat, FISHER AND PARSONS[48] observed a progressive diminution in the rate at which glucose moved from the mucosal into the serosal compartment with the distance of the segment used from the pylorus. With galactose, however, the middle third of the intestine was found to be most active[17]. As mentioned above, this apparent difference in localization led FISHER AND PARSONS to suggest that glucose and galactose are transported by different mechanisms or different cells. However, since the observations made by FISHER AND PARSONS with glucose diverge not only from their own results with galactose but also from those obtained in the present studies with glucose, galactose and 1,5-anhydro-D-glucitol, it would seem possible that the explanation for the divergence may be found in the characteristics of glucose transport *in vitro* rather than in the location of the active transport process. For instance, in the measurement of the glucose transferred in the *in vitro* preparation used by FISHER AND PARSONS[16] an appreciable quantity of endogenous glucose was formed during the experiment and this endogenous glucose contributed to the measured rate of glucose transport. Endogenous stores of glucose (glycogen) may be expected to be greater in the cells more proximal to the pylorus inasmuch as these are exposed during digestion to high luminal concentrations of glucose[49]. Also, utilization of glucose accounted for an appreciable fraction of the glucose which disappeared from the mucosal compart

ment in the experiments of FISHER AND PARSONS. It was assumed by these authors in their interpretation of the results that glucose which is transported enters the mucosal cells by a different portal than glucose which is metabolized.

If, on the contrary, it is assumed that all glucose enters the epithelial cells by the same portal and that the total glucose disappearance is equal to the rate of transport, the gradient calculated from the data of FISHER AND PARSONS[48] would be much less steep than the one presented. Experiments reported in a later paper of this series[18] show that transported sugar is accumulated within the epithelial cells and it seems reasonable to conclude that the same glucose pool contributes to diffusion into the submucosal tissues and to utilization. In experiments with galactose, the possible contributions of endogenous sugar and utilization to the measured rate of transport are greatly reduced, since there are no large tissue stores of galactose and galactose is not well-metabolized by the intestinal mucosa. It may be expected that the rate at which galactose is transferred will be more nearly representative of the rate of active transport by the epithelial cells.

Third, there is the question of the identification of the process by which sugar is accumulated within intact strips with the process by which sugar is absorbed from the intestine, *in vivo*, or transferred from the mucosal to the serosal sides of other types of *in vitro* preparations. It seems clear from the data presented in Tables V and VI that the accumulation of sugar by the intact strip is a property of the mucosal epithelial cells and that sugar appears in the underlying layers of the intestinal wall as the result of diffusion from the high concentration provided by the activity of the epithelial cells or by entrainment in the water stream[40]. Mucosal sheets and villi accumulate sugar to high relative concentrations—serosal sheets do not—and there is no reason to doubt that these studies on accumulation measure the same process that is measured *in vivo* and by other techniques, *in vitro*. By way of contrast, this is not so clearly the case with amino acids. Amino acids have been shown to be transported against an apparent concentration gradient by everted sacs of intestine[50,51] and it has seemed reasonable to assume that this is a property of the epithelial cells. However, accumulation of amino acids is a property of many cells[52,53] and the data presented in Table VI show that the intestinal musculature also possesses this property. These results would seem to leave in doubt the precise relationship of experiments on amino acid transport with excised intestinal tissue to amino acid absorption *in vivo*.

ACKNOWLEDGEMENTS

This investigation was supported by grant No. G-5892 from the National Science Foundation. One of us (P.M.) is a fellow of the National Foundation. The technical assistance of M. L. ROBERTS is greatly acknowledged.

REFERENCES

1 E. BARANY AND E. SPERBER, *Scand. Arch. Physiol.*, 81 (1939) 290.
2 P. N. CAMPBELL AND H. DAVSON, *Biochem. J.*, 43 (1948) 426.
3 R. M. ATKINSON, B. J. PARSONS AND D. H. SMYTH, *J. Physiol. (London)*, 135 (1957) 581.
4 R. B. FISCHER AND D. S. PARSONS, *J. Physiol. (London)*, 110 (1949) 36.
5 W. A. DARLINGTON AND J. H. QUASTEL, *Arch. Biochem. Biophys.*, 43 (1953) 194.
6 T. H. WILSON AND G. WISEMAN, *J. Physiol. (London)*, 123 (1954) 116.
7 R. K. CRANE AND T. H. WILSON, *J. Appl. Physiol.*, 12 (1958) 145.

[8] T. Z. CSAKY AND G. W. FERNALD, *Am. J. Physiol.*, 198 (1960) 445.
[9] T. ROSENBERG, *Symposia Soc. Exptl. Biol.*, 8 (1954) 136.
[10] E. RIKLIS AND J. H. QUASTEL, *Can. J. Biochem. and Physiol.*, 36 (1958) 347.
[11] T. Z. CSAKY AND M. THALE, *J. Physiol.* (London), 151 (1960) 59.
[12] R. K. CRANE AND S. M. KRANE, *Biochim. Biophys. Acta*, 20 (1956) 568.
[13] T. H. WILSON AND R. K. CRANE, *Biochim. Biophys. Acta*, 29 (1958) 30.
[14] R. K. CRANE AND S. M. KRANE, *Biochim. Biophys. Acta*, 31 (1959) 397.
[15] T. H. WILSON AND B. R. LANDAU, *Am. J. Physiol.*, 198 (1960) 99.
[16] R. B. FISHER AND D. S. PARSONS, *J. Physiol. (London)*, 119 (1953) 210.
[17] R. B. FISHER AND D. S. PARSONS, *J. Physiol. (London)*, 119 (1953) 224.
[18] D. B. MCDOUGAL JR., K. D. LITTLE AND R. K. CRANE, *Biochim. Biophys. Acta*, 45 (1960) 483.
[19] N. LIFSON AND D. S. PARSONS, *Proc. Soc. Exptl. Biol. Med.*, 95 (1957) 532.
[20] S. M. KRANE AND R. K. CRANE, *J. Biol. Chem.*, 234 (1959) 211.
[21] W. T. AGAR, F. J. R. HIRD AND G. S. SIDHU, *Biochim. Biophys. Acta*, 14 (1954) 80.
[22] W. T. AGAR, F. J. R. HIRD AND G. S. SIDHU, *Biochim. Biophys. Acta*, 22 (1956) 21.
[23] H. A. KREBS AND K. HENSELEIT, *Z. physiol. Chem. Hoppe Seyler's*, 210 (1932) 33.
[24] F. DICKENS AND H. WEIL-MALHERBE, *Biochem. J.*, 35 (1941) 7.
[25] A. S. KESTON, *Abstracts of Papers, Meeting, Am. Chem. Soc., April*, 1956, p. 31C.
[26] A. SAIFER AND S. GERSTENFELD, *J. Lab. Clin. Med.*, 51 (1958) 448.
[27] M. W. SLEIN, G. T. CORI AND C. F. CORI, *J. Biol. Chem.*, 186 (1950) 763.
[28] M. SOMOGYI, *J. Biol. Chem.*, 195 (1952) 19.
[29] Z. DISCHE AND L. B. SHETTLES, *J. Biol. Chem.*, 175 (1948) 595.
[30] J. H. ROE, J. H. EPSTEIN AND N. P. GOLDSTEIN, *J. Biol. Chem.*, 178 (1949) 839.
[31] H. T. MCPHERSON, *Biochem. J.*, 40 (1946) 470.
[32] K. E. WILZBACH, *J. Am. Chem. Soc.*, 79 (1957) 1013.
[33] E. HELMREICH AND R. K. CRANE, in F. TURBA, *Radioisotopes in Physiology, Diagnosis and Therapy*, Editor, Springer-Verlag, Berlin, in the press.
[34] R. K. CRANE, R. A. FIELD AND C. F. CORI, *J. Biol. Chem.*, 224 (1957) 649.
[35] C. R. PARK, R. L. POST, C. F. KALMAN, J. H. WRIGHT, L. H. JOHNSON AND H. E. MORGAN, *Ciba Colloq. on Endocrinol.*, 9 (1955) 240.
[36] R. B. FISHER AND D. B. LINDSAY, *J. Physiol. (London)*, 131 (1956) 526.
[37] R. HÖBER AND J. HÖBER, *J. Cellular Comp. Physiol.*, 10 (1937) 401.
[38] G. H. A. CLOWES, A. K. KELTCH, C. F. STRITTMATTER AND C. P. WALTERS, *J. Gen. Physiol.*, 33 (1950) 555.
[39] R. K. CRANE, *Biochim. Biophys. Acta*, 45 (1960) 477.
[40] R. K. CRANE, *Physiol. Revs.*, 40 (1960) 789.
[41] C. F. CORI, *The Harvey Lectures*, 23 (1927–28) 76.
[42] J. LARRALDE, *Rev. espan. fisiol.*, 3 (1947) 31.
[43] R. B. FISHER AND D. S. PARSONS, *J. Anat.*, 84 (1950) 272.
[44] T. H. WILSON AND T. N. VINCENT, *J. Biol. Chem.*, 216 (1955) 851.
[45] T. H. WILSON AND G. WISEMAN, *J. Physiol. (London)*, 123 (1954) 126.
[46] B. R. LANDAU AND T. H. WILSON, *J. Biol. Chem.*, 234 (1959) 749.
[47] D. SHACTER AND S. M. ROSEN, *Am. J. Physiol.*, 196 (1959) 357.
[48] R. B. FISHER AND D. S. PARSONS, *J. Physiol. (London)*, 110 (1950) 281.
[49] P. C. REYNELL AND G. H. SPRAY, *J. Physiol. (London)*, 134 (1956) 531.
[50] G. WISEMAN, *J. Physiol. (London)*, 127 (1955) 414.
[51] W. T. AGAR, F. J. R. HIRD AND G. S. SIDHU, *Biochim. Biophys. Acta*, 22 (1956) 21.
[52] D. D. VAN SLYKE AND G. M. MEYERS, *J. Biol. Chem.*, 16 (1913–14) 197.
[53] H. N. CHRISTENSEN, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, The Johns Hopkins Press, Baltimore, 1955, p. 63.
[54] J. P. KALTENBACH, M. H. KALTENBACH AND W. B. LYONS, *Exptl. Cell Research*, 51 (1958) 112.