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ABSTRACT

GLUCOSE ABSORPTION AND METABOLISM BY
THE GUT OF RAINBOW TROUT,
SALMO GAIRDNERI

by Robert Mitchell Stokes

An *in vitro* recirculation method was used to measure absorption and metabolism of D-glucose by trout intestinal segments. Mean rates of glucose absorption from mid-gut segments were 8.72 and 26.00 μg/mg dry wt/hr at 14 and 24°C, respectively, with an initial media glucose concentration of 50 mg/100 ml. Absorption rate was 65 to 85 per cent lower in caecal and large gut sections on both a tissue weight and surface area basis. Calculated active transport (mucosal absorption - 3/5 total glucose utilization) was approximately 30 per cent of mucosal absorption. Glucose absorption is increased greatly by increased media temperature, media glucose concentration, and feeding activity of the fish, whereas the rates of glycogenolysis, glycolysis, and oxidative respiration were relatively constant under the same conditions. Rates of all catabolic processes in those regions of the gut studied (caeca and mid-gut) were of comparable magnitude. Mucosal membranes restrict diffusion

of endogenous glucose and lactic acid into the surrounding media more than serosal membranes, e.g., during perfusion of mid-gut with media containing no initial substrate, averages of 65 and 94 per cent of the total glucose and lactic acid, respectively, entering mucosal and serosal media, appeared on the serosal side. Caecal and mid-gut segments from trout exposed to 2.5 mg/l chromium for 7 days, respectively absorbed 40 and 32 per cent less glucose than controls, whereas no significant effect on other metabolic processes could be shown.

GLUCOSE ABSORPTION AND METABOLISM BY

THE GUT OF RAINBOW TROUT,

SALMO GAIRDNERI

By

Robert Mitchell Stokes

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology and Pharmacology

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ACKNOWLEDGMENT

The author wishes to extend his appreciation to Dr. Paul O. Fromm, Department of Physiology and Pharmacology, for his guidance throughout this study, particularly for allowing freedom to develop and express independent ideas.

In addition the author is grateful to the United States Public Health Service, National Institutes of Health for contributing the funds to support this work.

Dedicated to my wife and our two children, whose sacrifices and encouragements made the completion of this work a reality.

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INTRODUCTION

Although considerable progress has been made in recent years on understanding membrane structure and permeability, elucidation of the physicochemical factors responsible for movement of materials across it are still largely unresolved. Transit may occur by either passive diffusion or through special mechanisms, such as carrier facilitated diffusion, solvent and ionic drag, pinocytosis, and active transport. The latter process, which is of concern in this paper, is distinguished from all others by two criteria: energy dependence and transport against a concentration difference.

The relative importance of each transport mechanism varies with the type of cell. For example, active transport of glucose by the intestinal mucosa is a unique process believed to be shared only with the functionally similar epithelium of the proximal convoluted tubule of the kidney (Crane, 1960). Evolution of nutrient transporting enzyme systems in these tissues makes it possible, at least in homeothermic or active animals, to maintain a high homeostatic level of select blood constituents for adequate cell nourishment.

This uniqueness of the mucosa has naturally led many t

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investigators to study this tissue in an attempt to characterize active transport as such, and more specifically how this process moves glucose. Mammals have been used primarily as experimental animals because of the direct application to human physiology, and reference to glucose transport in poikilotherms is extremely limited. Active glucose transport is known to occur in the intestine of cold-blooded animals and there is no reason to believe that the basic mechanism is different than that found in mammals. The study of poikilothermic tissues provides a means to examine active transport at a naturally reduced rate.

The basic purpose of this study was therefore to examine active transport of glucose by mucosal tissue of teleost fish as a representative poikilotherm in order to support proposed mechanisms of transit in mammals, to determine what differences occurred, if any, and to speculate on the importance of this process to the nutrition of the animal investigated. Phases of glucose translocation receiving particular attention were: extent and rate of movement with different states of nutrition and temperature, the influence of gut metabolism on transport processes, and localization of regions in the tract primarily responsible for active transport.

Knoll and Fromm (1960) have shown that the rainbow trout intestinal tract, particularly the pyloric caeca, is a prime target for hexavalent chromate ion accumulation. Hexavalent chromium is known to be toxic; the degree of toxicity depending on the amount of accumulation and duration of exposure. The etiology and pathogenesis of this action, however, is still unknown. In light of this unresolved question, it was of interest to evaluate the effects of this ion on an energy dependent, enzymatic process such as active glucose transport in the gut of fish exposed to chronic chromate levels.

LITERATURE REVIEW

The significance of literature concerning sugar absorption by the gastrointestinal tract has recently been interpreted and summarized in the extensive reviews of Crane (1960) and Wilson (1962). For proper orientation of the reader to evaluate the following study, certain general points concerning sugar absorption must be restated. More specific reference to pertinent studies on poikilothermic species will be given as they apply.

As early as 1900 it was recognized that the small intestine absorbed hexoses faster than pentoses. In 1925 Cori showed that the relative rates of simple sugar absorption in rat intestine were: galactose $>$ glucose $>$ mannose $>$ fructose $>$ pentoses. These rates have been confirmed not only in rats and other homeothermic animals, but also in frogs (Westenbrink and Gratama, 1937; Minibeck, 1939; Cordier and Worbe, 1954) and fish (Cordier and Chanel, 1953; Cordier et al., 1954). The criteria for absorption in most of these early qualitative *in vivo* studies was per cent sugar absorbed from sugar solutions placed into the lumen of the intact gut or ligated loops.

These observations resulted in the concept of "selective permeability" of the small intestine. It was not until the work of Barany and Sperber (1939) that the hypothesis of active transport was spawned. They observed continued disappearance of glucose from rabbit intestinal loop when the luminal concentration had dropped below that of the blood. In 1943, Csaky first illustrated that this disappearance is due to active mechanisms and not utilization alone by the epithelial cells in demonstrating movement of non-metabolizable 3-0-methyl-D-glucose against its own concentration gradient in rats. This substituted sugar is also actively transported by frog and toad gut (Csaky and Thale, 1960; Csaky and Fernald, 1960). With the recent development of suitable <u>in vitro</u> techniques, whereby concentration differences across the mucosa can be controlled, it has been conclusively demonstrated that glucose and galactose are the only naturally occurring sugars which are absorbed by mammalian gut against a concentration difference. Poikilotherm intestine appears to behave in the same manner when the same criteria and methods are used. Active glucose transport has been demonstrated in the turtle (Fox, 1961) and fish (Musacchia and Fisher, 1960) during <u>in vitro</u> incubation of everted (turned inside out) intestinal sacs a

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and in isolated tied sacs of toad and frog gut (Csaky and Thale, 1960; Csaky and Fernald, 1960). Failure to express data in terms of absorption or transport per unit tissue mass or area, failure to account for concurrent effects of water movement and sometimes poor control of other variables makes it extremely difficult to quantitate and compare these specific studies on cold-blooded animals.

In recent years energy-dependence has been used to differentiate active transport from all other membrane transport phenomena. As early as 1930, it was recognized that inhibition of energy-yielding reactions by anaerobic conditions and specific enzyme poisons such as iodoacetate and phlorizin would greatly decrease the rates of glucose and galactose absorption but not other sugars in kidney and gut. The inhibitory effects of anoxia have been demonstrated in fish and frogs by Cordier and collaborators (1953, 1956). Musacchia and Westhoff (1963), using everted sac techniques have recently illustrated phlorizin inhibition of sugar transport in scup and catfish gut, but transport was inhibited by induced anoxia only in the scup. Carlisky and Huang (1962) report inhibition of glucose transport across isolated sheets of dogfish shark mucosa by sodium azide, iodoacetate, uranyl acetate, and phlorizin, but not by 2,4-dinitrophenol.

Other experimental criteria, such as conformity to Michaelis-Menton kinetics, competitive inhibition of similar compounds for the same pathway, and high temperature coefficients (Q_{10}), have often been used to characterize active transport. Glucose and galactose absorption rates increase to a maximum (saturation phenomena) as the luminal sugar content is elevated, whereas, absorption rates of other sugars increase nearly proportionally with each rise in concentration. Although saturation phenomena suggest processes other than passive diffusion, at substrate concentrations below the maximum rate active and passive transport illustrate similar kinetics. Also kinetic conformity is not necessarily indicative of enzyme controlled reactions since any physical rate-limiting process, such as membrane adsorption, would exhibit a similar curve.

Only Carlisky and Huang (1962) have attempted a kinetic analysis of glucose absorption in poikilothermic animals. They measured absorption across isolated sheets of mucosa from dogfish shark, because transport could not be detected across the intact gut. Calculated Km values of 20 mM and 16 mM at 36 and 26^{o}C, respectively, are considerably higher than values reported for mammalian tissues under similar conditions. This indicates that glucose has less affinity for the transport system in the shark. However, since leaks were reported in the mucosal sheets, transport rates should be underestimated. Flow processes would passively counteract the consequence of glucose transfer to a submucosal fluid in a system where a sheet of tissue separates fluid of equal initial glucose content.

In general, high temperature coefficients have been associated with active transport. However, data must be interpreted with caution since high Q_{10} values have been observed for passive movement across the cell membrane. Temperature coefficients of about 2 have been observed by Cordier et al. (1954b, 1955) in the fish and in the frog (1954a) using *in vitro* gut sacs. Employing similar methods Csaky and Frenald (1960) show about the same Q_{10} for 3-0-methyl-D-glucose absorption in the frog. Rate of glucose transport from 26 to 36°C apparently increased 10 fold in dogfish mucosa (Carlisky and Huang, 1962). Fox (1961) found transport of glucose decreased to zero in everted sacs from cold-torpor turtles incubated at 2°C.

Although kinetic parameters mentioned above are definite characteristics of active transport they also can occur in another transport phenomenon termed facilitated diffusion. In this process the rate of diffusion equilibration is greatly accelerated, but it is energy-independent and will not move materials against an electrochemical gradient. The kinetics of facilitated diffusion and active transport suggest that a membrane carrier is involved.

Localization of glucose and galactose inside the epithelial cell indicates that the mechanism for active absorption is located in the apical or brush border region. McDougal et al. (1960) measured the D-galactose content of various layers of intestinal wall previously separated by microdissection after initial freeze-drying. They found highest sugar concentration in the epithelial cell. With less precise methods, glucose accumulation has been shown in frog mucosa (Csaky and Fernald, 1961). Concurrently in 1961 Kinter arrived at the same conclusion using autoradiographic procedures to localize galactose-C^{14} in the gut wall.

The occurrence of competitive inhibition between sugars which are actively transported suggests that they all enter a common transport pathway. Specificity studies have shown that these sugars have a common minimum structure (figure 1). The proposed common path appears to be separated into two steps: a carrier-mediated, <u>energy-independent</u> entrance into the cell and an <u>energy-dependent</u> movement against a concentration gradient. Crane (1962) has definitely shown that

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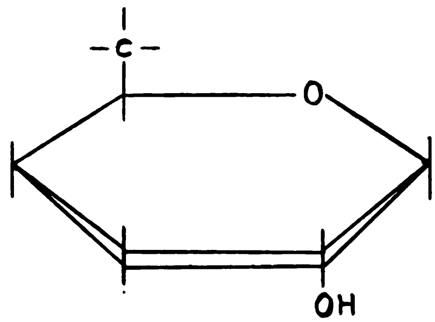


Figure 1. Minimum Structural Requirements for Intestinal Transport of Sugars (Crane: Physiol. Rev., 40, 1960)

accumulation of glucose in epithelial cells is not dependent on an energy supply. Newey et al. (1959) demonstrated that phlorizin (at concentrations below which it effects endogenous respiration) inhibited sugar entrance into the cell from the mucosa side, while utilization from the serosa side is uneffected. From these results, it could be postulated that initial transport steps follow the characteristics of facilitated diffusion and a later process accounts for active transport.

Various chemical reactions have been proposed to account for the energy-dependence of active transport. The best known is the phosphorylation-dephosphorylation concept originally developed by Hober and Verzar (Wilson, 1962). The enzyme hexokinase was believed to catalyze phosphorylation of glucose to glucose-6-P, with subsequent dephosphorylation by non-specific phosphatases after transit through the epithelial cell. This theory no longer appears to be valid.

The strongest contrary evidence is the fact that only the C-2 hydroxyl is necessary for active transport (figure 1) and phosphorylation at this position is not consistent with the capabilities of known kinases. Also, Landau and Wilson (1959) found that less than 10 per cent of absorbed glucose goes through the glucose phosphate pool.

Formation of lactic acid during glucose absorption has raised the possibility of triose phosphate production followed by recondensation to hexose as a transport avenue. The lack of randomization of labeled glucose during transport is major evidence against this hypothesis. In 1954, Keston presented evidence for the direct involvement of mutarotase in active absorption, but the fact that certain actively-moved sugars will not mutarotate makes this theory unlikely.

If chemical conversion is involved it should occur at the C-2 position. Crane and Krane have eliminated hydrolysis, since O^{18} does not exchange between water and sugar hydroxyls. They also dismissed oxidation-reduction after continued active transport of synthetic sugar which contained a hydroxymethyl group substituted for hydrogen at C-2.

In view of the evidence presented, one must postulate that the cell provides energy for active translocation by some less direct means. Recent data hint that sodium transport may be the second energy-dependent system with which active sugar movement couples. Riklis and Quastel (1958) and Csaky and Thale (1960) have shown that glucose absorption is dependent on sodium in the bathing media of guinea pig and frog gut. Crane, Miller, and Bihler (Crane, 1962) report a direct correlation between Na content of media and active sugar transport. Digitalis, a known inhibitor of Na transport, likewise inhibits active sugar transport (Csaky et al., 1961).

Crane (1962) points out that this suggested coupling of transport systems does not appear to involve transcellular movement of Na from gut lumen to blood. Data presented by Crane (1962) also show that uptake of glucose by the cell is dependent on sodium, under conditions which abolish active glucose transport, due to a reduction of energy supplies. In light of the apparent Na-dependence for the entire sugar transport process and evidence for an energy-independent-dependent two phase absorption mechanism, Crane has proposed an interesting hypothesis: Phase I -Na and glucose moving into the epithelial cell associate with a mobile carrier; this complex traverses the apparent diffusion barrier; Na and glucose are released to inner cytoplasm. Phase II - Na is returned to medium by the "sodium-potassium pump"; glucose remains due to lack of Na for its return to the medium by the carrier complex. The energy-dependent return of Na facilitates free glucose accumulation, necessarily against its own concentration difference.

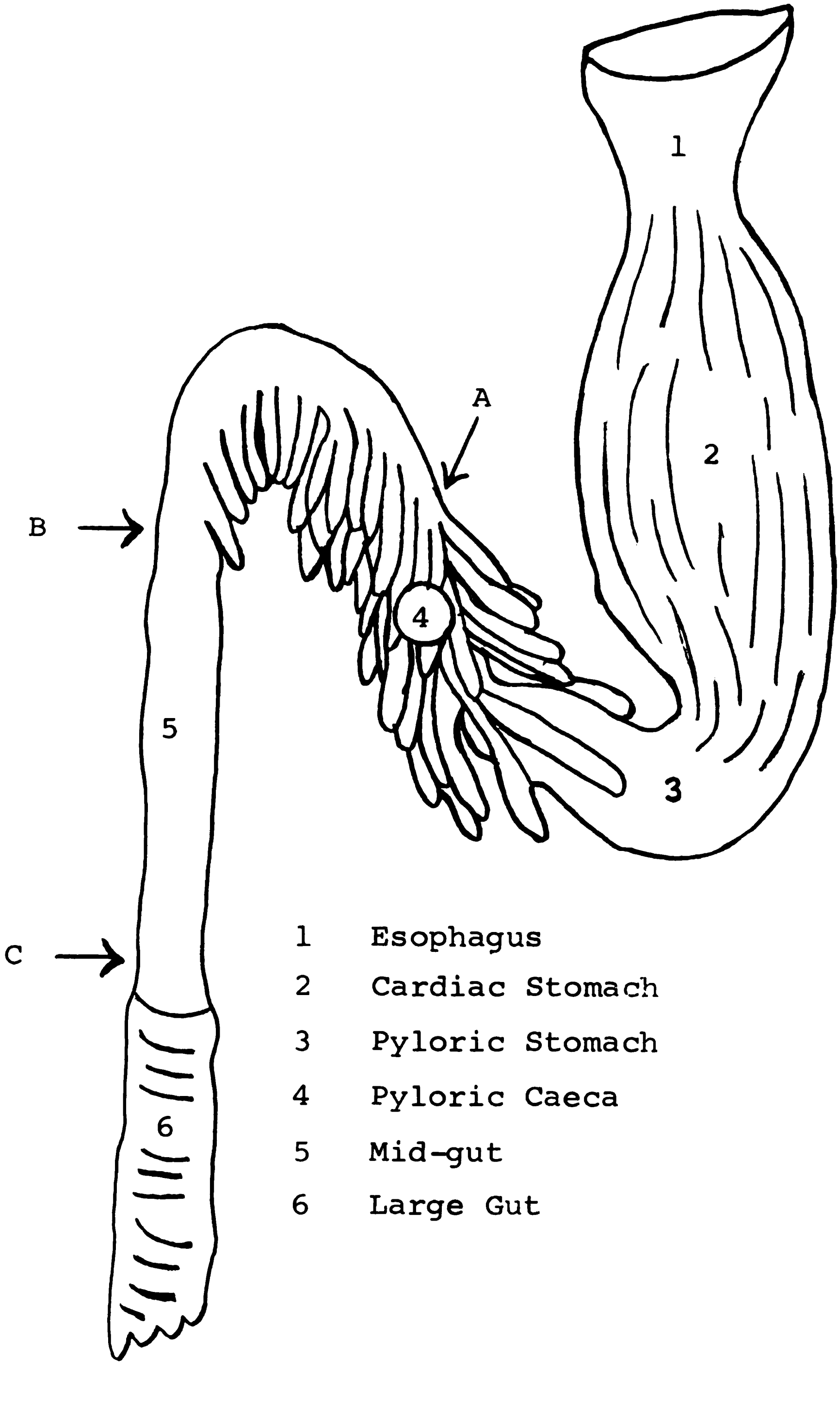
MORPHOLOGY AND HISTOLOGY OF GUT

Figure 2 illustrates the gross anatomy of the rainbow trout gut. The short esophagus can be divided into four radial layers: (1) columnar epithelium with mucous cells, (2) sub-epithelial connective tissue, (3) thick, circular, striated muscle, and (4) a thin serosa. Salmonid fish are physostomatous and the pneumatic duct enters the posterior esophagus (Burnstock, 1959).

The stomach is looped into descending cardiac and ascending pyloric regions. Burnstock reports that brown trout have no cardiac sphincter, only a transitional zone where typical stomach layers begin, including a smooth muscle coat. A pyloric sphincter is present. The layers of the stomach wall are: (1) columnar epithelium, folded to form gastric pits, (2) tunica propria, which encloses serous and mucous glands, respectively, located at the base of the gastric pits in the cardiac and pyloric regions, (3) stratum compactum, (4) stratum granulosum, (5) muscularis mucosa, (6) submucosa, (7) smooth muscle layers with the circular coat being more than twice the thickness of the longitudinal coat, and (8) a serosa (Weinreb and Bilstad, 1955; Burnstock, 1959).

Figure 2

Gross Anatomy of Trout Gut, Showing Regions
Studied (A-B = Caecal Segment;
B-C = Mid-gut Segment)



1 Esophagus
2 Cardiac Stomach
3 Pyloric Stomach
4 Pyloric Caeca
5 Mid-gut
6 Large Gut

The intestine consists of short ascending and long descending portions. Finger-like pouches, pyloric caeca, project from the ascending and upper descending arms and are reflected backward toward the stomach. In the forward half of the ascending part, caeca project peripherally from most of the main tract wall; in the remaining portion they extend from the posterior-ventral side only. A thickening of circular muscle, which may act as a sphincter, is found at the mouth of each caecum (figure 3). In 14 animals, the average number of caeca was 67 (range 52-92).

The liver overlies the descending stomach region and curve of the intestine. The common bile duct follows the hepatic portal veins and hepatic artery and enters the main intestinal tract slightly posterior to the pyloric sphincter. Weinreb and Bilstad (1955) also report that the pancreatic duct joins the caeca tract adjacent and posterior to the bile duct. Pancreatic tissue in the trout is diffuse and lies mainly between the folds of the pyloric caeca. The mesentaries enclosing the caeca are sites of fat deposition.

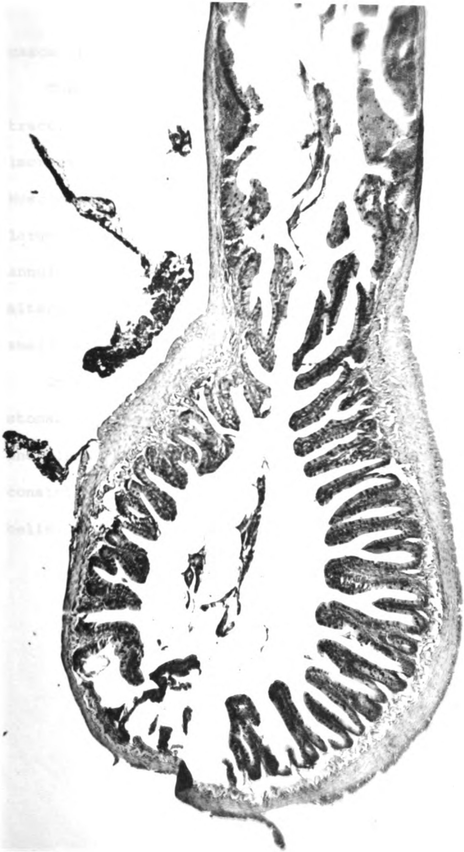
A section of intestine 3 to 4 cm long (body weight less than 200 grams), lying posterior to the caeca region and anterior to the large gut, is referred to as mid-gut. This region is histologically similar to the region of pyloric

Figure 3

Transverse Section Through Pyloric Caeca Region

at Its Junction with a Single Caecum

(H X E 50 X)



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caeca (figure 3; figure 4).

The rectum or large gut comprises the remainder of the tract. At the origin of this portion, the gut wall abruptly increases in diameter and appears more darkly pigmented. Muscle layers progressively thicken toward the anus. The large gut mucosa is folded in such a manner as to produce annulo-spiral septa (Burnstock, 1959). This morphological alteration resembles the folds of Kerkring in the human small intestine.

Intestinal histology (figure 5) differs from that of the stomach by the absence of a submucosa, muscularis mucosa, and glands in the tunica propria. The surface epithelium consists of columnar cells with a striated border and goblet cells.

Figure 4

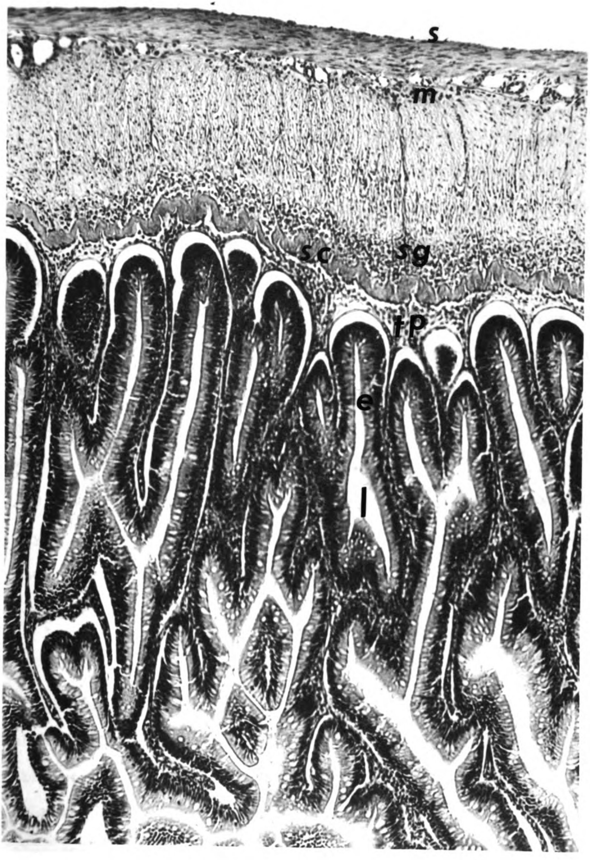
Transverse Section of Several

Individual Caeca

(H X E   140 X)

Figure 5

Longitudinal Section Through Wall of Mid-gut. e, Mucosal Epithelium; tp, Tunica Propria; sc, Stratum Compactum; sg, Stratum Granulosum; m, Smooth Muscle; s, Serosa; l, Lumen (H X E 140 X)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METHODS

Experimental Animals

Rainbow trout (Salmo gairdneri) were selected as experimental animals because they are poikilothermic, have interesting morphological variations of the gastro-intestinal tract such as pyloric caeca and are available as needed from the Michigan Department of Conservation Wolf Lake Hatchery. Trout, ranging from 7 to 12 inches, were transported from the hatchery in a galvanized metal tank lined with non-toxic paint and fitted with an agitator for aeration. They were held in a constant temperature room in fiberglass-lined wooden tanks under continuous illumination at $12 \pm 2^{\circ}C$ and fed commercial trout pellets *ad libitum* once on alternating days. Water was changed at two-day intervals. It was found that approximately two weeks were required for the trout to adjust to their new environment and resume feeding activity.

Justification of Method

As described earlier, a profusion of methods are available to the experimenter interested in studying intestinal absorption. *In vivo* techniques have an important advantage of being more physiological. However, determination of absorption rate without concurrent measurement of blood levels anc

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and/or appropriate corrections for metabolic losses will yield an overestimation of available nutrient to the remainder of the body tissues. Measurement of absorbed material in the blood would be difficult to achieve because of small portal vessels and blood volume in the size of fish that were available. Data to account for metabolic losses may be obtained equally well *in vitro*. Also with *in vivo* methods the control of certain experimental variables, such as maintenance of equal or higher concentration of sugar on the tissue side of the luminal mucosal membrane is more difficult. This criterion is very important in distinguishing active transport from other modes of diffusion or transport since only this process can move materials against a concentration difference. The desired chemo-gradients are established in an *in vitro* preparation by separating solutions of desired solute content by the gut wall, whereas complicated intestinal and vascular perfusion techniques would be necessary to accomplish this task *in vivo*.

The *in vitro* techniques that have been used for absorption studies fall into five major categories: (1) sheets of intestine separating two fluid filled chambers, (2) incubation of tied segments, (3) everted sacs, (4) perfusion

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or recirculation, and (5) sugar accumulation in rings of everted intestine, mucosa sheets, and separated villi. The first two are considered unsatisfactory because of inadequate oxygenation of the epithelium. Eversion of segments, tied to form sacs, enables the mucosa to be in contact with continuously oxygenated media. Also this method is simple, requires little equipment, and is highly sensitive, since minute quantities of solute transported across the mucosa can be concentrated in a small serosal volume. Certain modifications of this method would have been acceptable for trout gut except that the morphological variations of the pyloric caeca made such an operation impossible, and comparison of different gut regions using the same techniques is imperative. This latter requirement was best met with a recirculation method, modified to minimize hydrostatic pressure gradients across the mucosa, to reduce possible flow damage to the tissues, and to allow rapid and simple attachment of the gut by specially constructed tissue reservoirs.

In tissue accumulation methods, the rate of sugar uptake by the tissue should more closely represent a true in vivo rate, since the serosal barrier is either removed or not considered. The existence of this procedure was unknown to the author until a good portion of the study had been completed. The reader is referred to recent reviews by Crane (1960) and Wilson (1962) for more detailed descriptions of past and present methods.

Apparatus

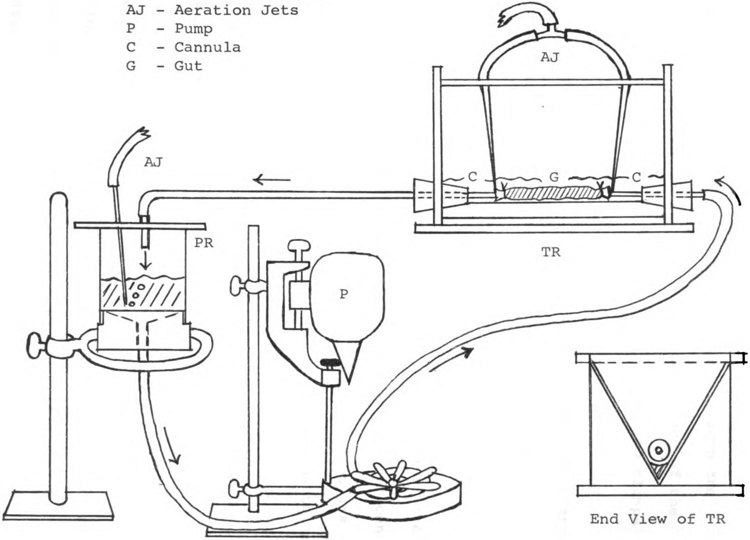
The recirculation apparatus (figure 6) consists of a V-shaped, plexiglas reservoir with openings in either end to accommodate rubber stoppers pierced by stainless steel cannulae. Cannulae were truncated 12 gauge hypodermic needles with grooves filed into the shank to allow firm attachment of the tissue. A section of intestine is suspended between the two cannulae and covered with media to bathe serosal surfaces. The same medium, circulating through the inside of the gut empties into a perfusate reservoir from which it drains and is subsequently returned to the gut through gum rubber tubing by a Kinetic Clamp pump (Sigmamotor, Inc., Middleport, N. Y.) driven by a Cone Drive stirring motor. This pump allows slow rates of flow; 6 cc/min was maintained in all experiments.

Hydrostatic pressure was equalized by the following procedure: Two 22 gauge hyperdermic needles were connected to the ends of a small section of capillary tube by small diameter rubber tubing and the system filled with fluid and

Figure 6

Perfusion Apparatus for Trout Gut Segments

PR - Perfusion Reservoir
TR - Tissue Reservoir
AJ - Aeration Jets
P - Pump
C - Cannula
G - Gut



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clamped at both ends to prevent entrance of air. A tiny air bubble was injected into the capillary fluid to serve as a marker. One needle was inserted vertically into the gut lumen and the other was placed under the surface of the serosal solution at an equal depth. After the tubing clamps were released the elevations of perfusion and tissue reservoirs were adjusted until the marker remained stationary for a period of 30 minutes. Since the perfusion pump delivers a pusatile flow, the mean luminal pressure was adjusted to the constant serosal pressure.

Using glass pipettes tissue and perfusate reservoirs were aerated at a constant rate by a 95 percent oxygen - 5 per cent carbon dioxide gas mixture during all experiments. This gas was originally selected to maintain the pH of a bicarbonate buffer. However, this buffer could not be used in manometric measurements of tissue respiration (see Tissue Respiration Methods) because of the necessary inclusion of a carbon dioxide absorber in the respiration flasks.

The temperature of incubation fluids was maintained by conducting the experiments in a constant temperature room. Plexiglas lids and high room humidity helped to reduce evaporative losses.

The entire apparatus was duplicated so that various sections of intestine from the same animal could be compared concurrently under similar conditions. Comparison could then be made without the influence of variation between individuals.

Tissue Medium

The medium (Table I) prepared for bathing rainbow trout tissues was modified from Burnstock's (1958) saline for brown trout. The major alteration was to increase the osmolarity of the more dilute Burnstock media to a value approximating that of trout plasma by decreasing original solvent volume. Relative proportions of Na, K, and Mg salts remained the same in the modified medium; only the absolute concentration was elevated. Careful adjustment of the Na concentration to plasma levels was of primary importance, since active transport of glucose is dependent on the presence of this ion (Csaky and Thale, 1959). The literature remains inconclusive about the effects of K and Mg.

It was also necessary to reduce the Ca ion content of the modified medium to a level whereby additions of phosphate buffer would not exceed the solubility product of the two ions in the final mixture. Bicarbonate buffer could not be employed in tissue respiration studies. Higher

NaC

KCl

CaC

MgS

NaH

KH_2

NaH

Na_2

Non

Na

K

Ca

Mg

TABLE I

PHYSIOLOGICAL SALINE FOR TROUT

Values Given in Grams per Liter as Anhydrous Salts

	Burnstock's Brown Trout Saline (1958)	Modified Rainbow Trout Medium	Holmes & Stott (1960) Medium
NaCl	5.90	7.37	7.41
KCl	0.25	0.31	0.36
$CaCl_2$	0.40	0.10	0.17
$MgSO_4$	0.14	0.18	0.15
NaH_2PO_4	-	-	0.40
KH_2PO_4	1.6	0.46	-
$NaHCO_3$	2.1	-	0.31
Na_2HPO_4	-	2.02	1.60
Nonelectrolytes:			
glucose	2.0	.50	
inulin		.36	
bacteriostat	------------	100 mg/ml streptomycin sulfate 50 units/ml Penicillin	

ION COMPOSITION OF MODIFIED TROUT SALINE

Values as mM/l

Na	155	Cl	132
K	7.7	PO_4	17.7
Ca	0.9	SO_4	1.5
Mg	1.5		

calcium levels were sacrificed for the desirability of using the same media in both perfusion and incubation experiments.

Glucose was present in concentration of 50 or 100 mg % when added. The lower concentration was initially employed because of close comparison to blood glucose levels of rainbow trout in our holding tanks. These concentrations are also low enough to prevent establishment of excessive initial osmotic gradients between extra- and intracellular fluids.

Inulin was initially included as a non-transportable constituent capable of indicating water movement by its subsequent concentration or dilution. A common tissue culture antibiotic was added to inhibit utilization of metabolic substrates by contaminating microorganisms of the gut.

To avoid bacterial growth prior to experimentation, stock solutions of the following: (1) Na, K, and Mg salts, (2) $CaCl_2$, (3) phosphate buffer, (4) inulin, and (5) bacteriostat, were prepared and added together a short time before each experiment. Glucose was added in solid form. Fresh medium was prepared for each experiment.

The resulting osmolarity of the final mixture averaged 277 (SD $\pm$ 5) mOs/l. This compared closely to an average plasma tonicity of 293 (SD ± 5) mOs/l (n = 6). Flame photometric analyses of plasma and medium Na, K, and Ca concentrations also indicated close agreement of principal solute content. As expected, plasma Ca was higher than medium Ca. Actual ionic levels may be about the same, however, because a large fraction of total blood calcium is often bound to organic constituents (Lockwood, 1961). Some time after the preparation of rainbow trout saline, it was noted that a similar medium had been prepared for the cutthroat trout, Salmo clarkii by Holmes and Stott (1960). This saline is also listed in Table I to illustrate how closely it compares to the rainbow trout medium.

An optimum concentration of ions in any prepared medium is difficult to achieve and depends largely upon what biological aspects the investigator wishes to study. Maintenance of tissue viability was of principal importance in this study. The prepared media appeared to accomplish this purpose for the duration of the experimental period. Figure 16 indicates that tissue respiration remained constant for at least four hours at 24°C, which in terms of time and temperature is the most stressing situation encountered. The few ion analyses made failed to show any alteration of potassium content in the medium after four hours. Potassium concentration might be expected to increase if major cell destruction was occurring.

Histological examination of the intestine perfused two (figure 7) and four hours (figure 8) at 24°C indicated that little if any, damage occurred after two hours but some appeared to have occurred after four hours. Other observations (not illustrated) showed tissues exposed for four hours at 14°C did not appear different from controls. Detachment of the epithelium from the stroma occurred even in control tissues (figure 5). This apparently is a fixation artifact caused by agonal contraction of the smooth muscle in the villi core (Maximow and Bloom, 1948). The stress of fixation itself probably magnified the apparent damage and although autolysis appeared to occur during prolonged perfusion, it was considered negligible.

Cannulation and Perfusion Procedure

Trout were removed from holding tanks and brought to the constant temperature room containing the perfusion apparatus. In studies at 14°C animals were immobilized immediately since experimental and holding temperatures were about the same. During 24°C studies the temperature of the water containing the fish was allowed to warm up to at least

Figure 7

Transverse Section of Mid-gut

After 2 Hours Perfusion

(H X E   140 X)

Figure 8

Transverse Section of Mid-gut Segment

After 4 Hours Perfusion

(H X E 140 X)

$20^{\circ}C$ before operation.

All trout were immobilized by a blow to the head, weighed, and opened by a mid-line abdominal incision to expose the viscera. Caecal sections were cannulated prior to the gut since they required more time for preparation. This enabled perfusion of both sections to begin about the same time.

Figure 2 shows the portion of caeca that was used in this study. More anterior sections were avoided because of confluence of bile and pancreatic ducts with the intestinal tract. Ligation was routinely performed on these ducts to insure against leaks, should they be included in the sections to be perfused.

The proximal ingoing cannula for the caecal section was inserted and securely tied to the intact tract and the tract was then cut away. Since pyloric caeca overlie the tract anteriorally it was necessary to include a certain amount of non-perfused, extraneous tissue around the proximal cannula to prevent severing caeca close to the ligature. The caecal section was then cut away from the tract approximately 0.5 cm below the last caecum. After flushing the lumen and rinsing the serosa with the perfusate the caeca segment was mounted onto the outgoing cannula which was stationary in the apparatus.

In all experiments the volume of perfusate (mucosal fluid) was 5 ml and serosal solution was 10 ml. Small enough volumes to indicate changes of solute concentration are desirable so long as they are large enough to cover the tissue and fill enough of the perfusate reservoir to prevent the pumping of air bubbles. Initially, both fluids contained the same solute concentration. Prior to experimentation, the bathing media was aerated and allowed to equilibrate to experimental temperatures.

After mounting the caecal tissue and filling the chambers with fluid, circulation was begun. The time necessary for this operation was approximately 15 minutes. An additional 6 to 8 minutes was necessary to cannulate, flush, and mount the mid-gut section on the duplicate apparatus. Time zero was recorded when perfusates initially entered the gut lumen. To obtain measurable transport at 14^{o}C experiments were continued for 4 hours. The same period of time was used for studies at 24^{o}C.

At the end of the perfusion period, mucosal and serosal solutions were drained into graduates for volume measurements and corrected for adherence losses (about 0.5 ml). Samples were placed into plastic vials and immediately stored in the freezer for future analyses. Mid-gut and caecal sections were removed from the cannulae and lengths and weights of portions of the intestine between ligatures were recorded. In the case of caecal segments, it was also necessary to measure the number of caeca being perfused and, in later experiments, the weight of extraneous tissue not being perfused. Dry weights were obtained as described in the section on Tissue Respiration methods. Finally the entire tissues were placed into potassium hydroxide for glycogen determinations.

For an estimate of intestinal glycogen content at the beginning of perfusion, tissue was removed and placed into KOH immediately after the mid-gut was mounted in the apparatus. Since it was necessary to use all the mid-gut tissue for transport studies, an initial glycogen sample was taken from either caecal or large gut regions. Investigations of intestinal glycogen content in control animals indicated that glycogen content per unit dry weight increased from pyloric caeca distally to anus (figure 15). The gradient was quite consistent within individual fish and the ratio of mid-gut to caeca glycogen content (mg/gm dry weight) was found to be 1.23. Large intestine was not selected for these calculations because of a proportionally greater variability in initial glycogen concentration.

In several experiments change in rate of glucose transport during the 4 hour period was determined. It was assumed that 0.1 ml aliquots taken at hourly intervals would not noticeably alter the final glucose content, however, correction was always made for total volume removed.

Analytical Determinations

Glycogen was measured according to the method of Montgomery (1957). Glucose was analyzed by the glucose oxidase method (Huggett and Nixon, 1957) using commercial "Glucostat" preparation (Worthington Biochemical Corporation, Freehold, N. J.). Glucose determinations were preceded by protein precipitation using approximately 2 per cent solutions of zinc sulphate and sodium hydroxide, equal volumes of which titrated to pH 7.0. Lactic acid analysis was done according to Barker and Summerson (in Hawk, Oser, and Summerson, 1954). Inulin determinations were modified from original procedures of Roe, Epstein, and Goldstein (Smith, 1957). Proteins were not precipitated for this procedure, since no significant difference occurred between treated and untreated samples. Also precipitated samples were more variable, presumably due to the additional pipetting involved. Osmolarity of solutions was determined using a Model C Fiske Osmometer with small sample adapter. Total osmolar concentrations were determined from a standard curve based on pure sodium chloride solutions.

Tissue Respiration

Tissue respiration was determined with a multiple-unit constant-temperature microrespirometer designed by Reineke (1961). Gut segments were split open, weighed, and incubated in Warburg type flasks containing 2 ml of trout saline plus 50 mg/100 ml glucose with KOH in a center well. Each flask contained less than 300 mg (wet weight) of tissue. Separate tissue samples were dried to constant weight at 95^{o}C to determine percentage dry matter. Incubation temperatures were 14 and 24^{o}C, maintained by a constant temperature room.

X-rays

Sections of pyloric caeca were perfused as previously described with a 20 per cent sodium iodide contrast medium. Immediately after the solution had passed through the tract the tubing joining both cannulae were clamped; the ingoing first to prevent pressure increase. Treated sections, along with a control segment perfused with only trout saline, were x-rayed immediately.

Surface Area Measurements

An estimate of relative surface area per unit length of mid-gut and pyloric caeca sections (taken from the location used in perfusions) was calculated using the following formulae:

1) Mid-gut SA = Mean Mucosa Circumference (MC) in mm X Mean Mucosa Length (ML) in mm per cm Serosal Length (SL).

2) Pyloric Caeca SA = [MC Caeca Tract in mm X ML Caeca Tract in mm per cm SL] + (MC Caecum in mm X ML Caecum per mm Caecum SL X Mean Caecum SL in mm X Mean Number of Caeca per cm Caeca Tract SL)

SA = Surface Area (cm^2)/cm Length of Intestine

Measurements of circumference and length were made of histologically prepared transverse and longitudinal sections of mid-gut, caeca tract, and individual caeca (see figure 9).

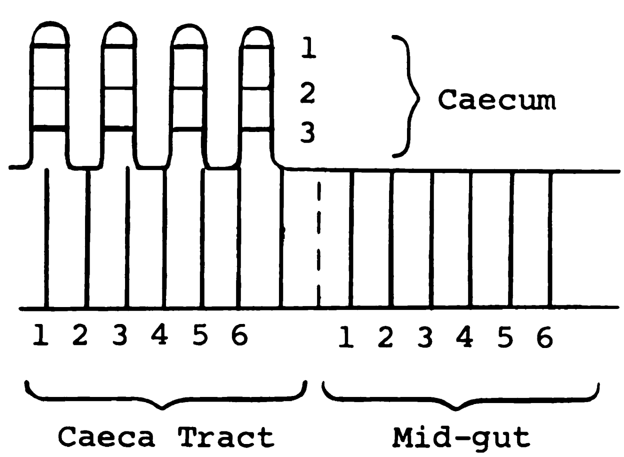


Figure 9. Location and Number of Histological Sections for Surface Area Measurements

The caecal sections represent 3 locations on 4 caeca. Measurements were repeated for 4 fish. Slides along with a scale for magnification corrections were projected on a screen, and dimensions were traced with a rotometer calibrated to read in the units projected. All caecal parameters, including mean length and number of caeca, were obtained from the posterior portion of the pyloric caeca region. See Table A for results of surface area measurements.

Measurement of Water Movement

During initial experiments, dramatic, unrealistic volume changes were evident when calculated on the basis of inulin concentration. The inulin concentration appeared to decrease in both serosal and mucosal solutions, however, it was consistently lower in the mucosal fluid. This effect could be produced by movement of fluid into the mucosa compartment or transport of inulin to serosal fluid. Another alternative is preferential degradation of inulin in the mucosal solution with either less inulin destruction in serosal fluid or water movement from mucosal to serosal fluids. Since inulin is not transported by the gut (Wilson, 1962) and water secretion is unlikely, the hypothesis for degradation was investigated.

Intestinal tissues were incubated in perfusion medium for 4 hours at 14°C. Less inulin was found at the end of the period in all incubation flasks, including blanks (significant at the 10 per cent level). Loss of inulin from blanks indicated tissue was not primarily responsible. If the tissue is not responsible for depletion then transport of inulin can not occur, for in order to be transported a material must initially be absorbed.

These observations supported suspicions that microbial growth was occurring. It had been observed earlier that the inulin content in refrigerated standard solutions decreased to two-thirds of the original in a period of one week. An exogenous source of contamination was indicated by depletion of inulin in blanks and standard solutions; contamination in intestinal contents is indicated by preferential mucosal depletion in perfusion studies.

In light of these suspicions, all inulin solutions were then autoclaved when prepared and discarded after one week and a general antibiotic was routinely added to the medium (see Table I). In subsequent perfusion experiments at 24°C, variability was reduced. However, the increase and decrease of inulin content in serosal and mucosal fluids, respectively, still occurred, giving continued support for the idea of net water movement into the gut lumen.

Comparison of inulin calculated and directly measured volumes showed the latter to be less variable. Direct volume measurements showed a slight mean volume decrease of approximately 1 per cent on both sides. Much of the disparity encountered with inulin probably resulted from the insensitivity of the method itself. For example, a difference of one per cent transmission on the colorimeter would represent a difference of 0.5 ml in calculated water transport.

Further investigation into sources of error was not attempted, since both methods showed water flux was not sufficient to appreciably alter glucose concentration. Direct volume measurements were subsequently adopted because of simplicity.

Chromium Methods

Caeca and mid-gut segments were removed from trout kept one week in aquaria containing 2.5 mg/l chromium as K_2CrO_4 and perfused as previously described. Knoll and Fromm (1960) have observed that caecal uptake of chromium, in trout exposed to the same concentration, was approaching a maximum after 1 week exposure. As a control, simultaneous experiments were conducted on fish maintained in tap water for the same duration. Both groups of animals were fasted, beginning 1 week prior to chromium exposure to allow complete emptying of the intestinal tract.

INTERPRETATION OF ACTIVE TRANSPORT

Primary criteria for quantification of active transport, using modern *in vitro* methods, have been rate of disappearance of sugar from fluids exposed to the mucosa, appearance in serosal solutions, or both. Rate of mucosal disappearance (or the less quantitative expression of per cent absorption) alone always represents an overestimation of the true quantity of sugar moving from gut lumen to blood, unless corrections are made for metabolic conversion and losses. This is an extensive task and is seldom attempted.

Sugar appearance or net gain in serosal fluids is believed to be a final product of active processes in the mucosa. However, since serosal and muscle tissue barriers tend to restrict sugar movement into serosal fluid, and the tissue itself will utilize sugar from serosal fluid, the true *in situ* transport is underestimated. For a more critical analysis, simultaneous determination of sugar fluctuation in both bathing fluids is imperative, not only to demonstrate actual existence of active transport, but to obtain a reasonable estimation of tissue utilization. This latter parameter is a prerequisite for accurate interpretation of the actual amount of sugar transferred by active transport.

The more critical approach was adopted for the trout studies with elimination of an initial concentration gradient across the mucosa, as previously described. Preliminary measurements of fluctuations of glucose content in mucosal and serosal fluids verified large reductions of mucosal fluid glucose. However, contrary to an expected rise, serosal glucose content was often lower than its initial content (figure 10). Serosal depletion was always minor and a serosal > mucosal glucose concentration gradient was consistently established. The following scheme for analysis of transport by trout gut *in vitro* was designed:

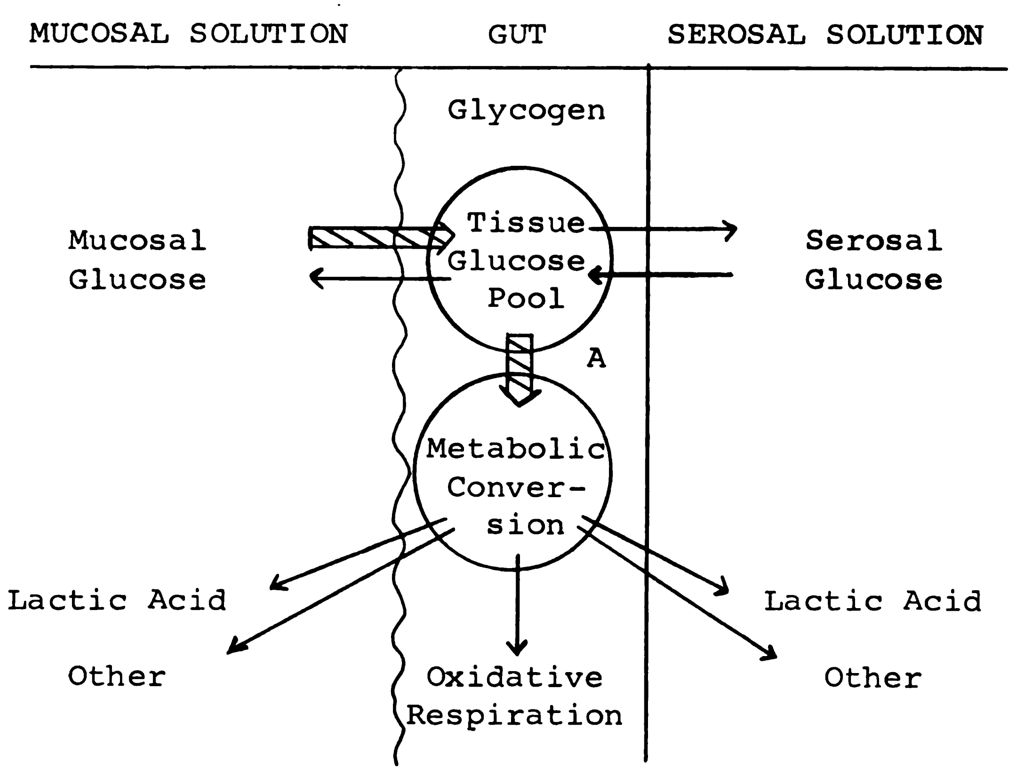
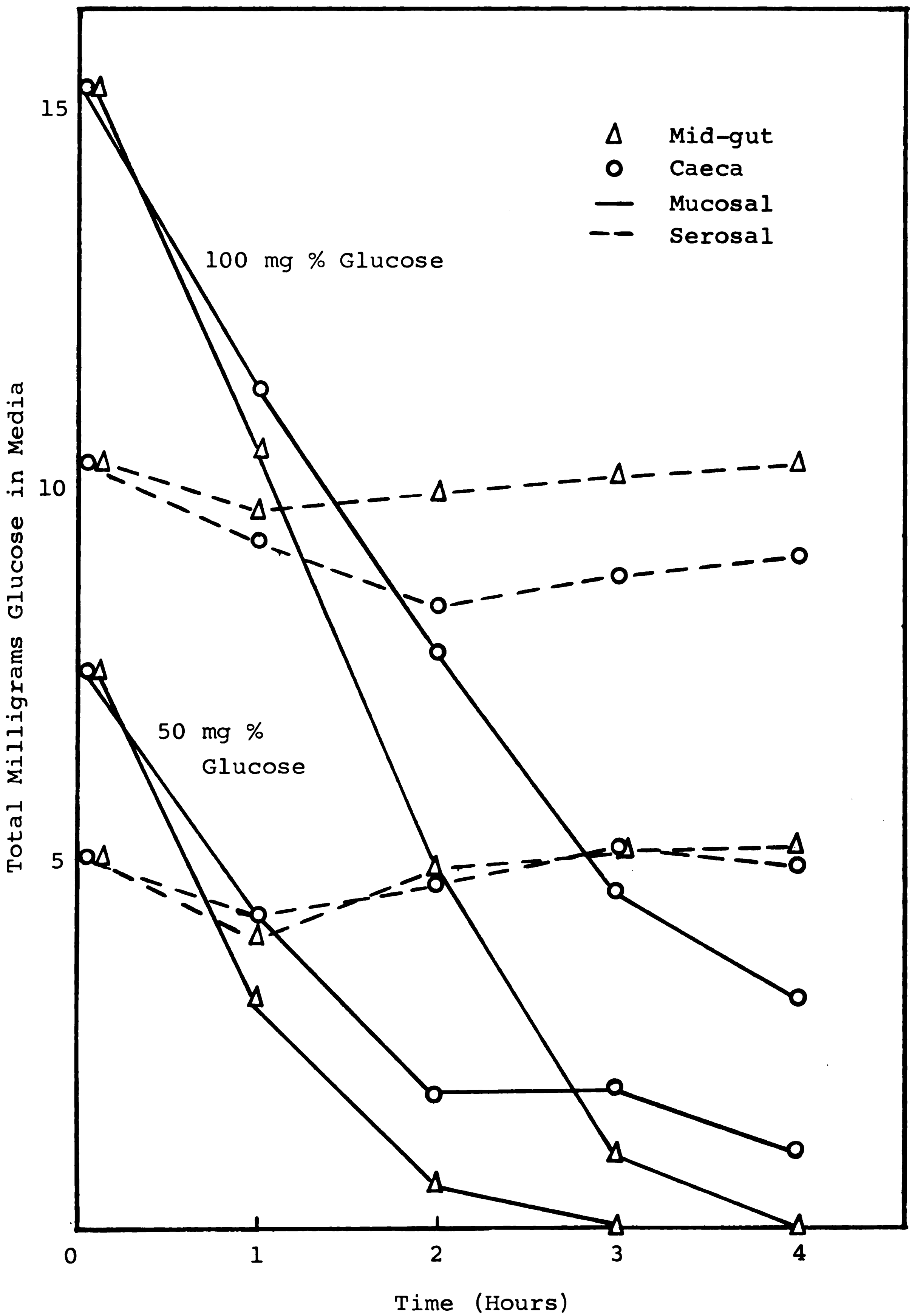


Figure 11. Hypothetical scheme for glucose metabolism during perfusion, *in vitro*.

Figure 10

Alteration of Glucose Concentration in Mucosal and Serosal Solutions During 4 Hours Perfusion at 24°C. Initial Concentration of Glucose on Both Sides Equaled 50 and 100 mg/100 ml in Separate Experiments



15
10
5
0
1
2
3
4
Total Milligrams Glucose in Media
Time (Hours)
Δ Mid-gut
O Caeca
Mucosal
Serosal
100 mg % Glucose
50 mg % Glucose

Total glucose available to the gut is represented by initial amounts of glucose contained in mucosal and serosal fluids plus glucose added by glycogen depletion or minus glucose used in glycogenesis. The initial glucose content minus glucose remaining in the media at the end of the perfusion period represents the total fraction utilized (arrow A, figure 11). Free glucose remaining in the tissue was assumed to be minor. Glycogen was selected as the sole indicator of endogenous carbohydrate because it was believed to be the major storage form. It was necessary to use the entire perfused segment for analysis in order to obtain a measurable quantity of tissue glycogen.

By multiplying per cent glucose utilized times the initial glucose content in mucosal and serosal fluids, it was possible to calculate a hypothetical value of glucose remaining on each side after the perfusion period if no active transport had occurred. The final measured glucose was always less than the calculated amount in mucosal fluids and was more by the same amount in serosal fluids. Either the negative mucosal or positive serosal value could be used as the true quantity of glucose actively transported.

Results indicate that 60 and 40 per cent of the total fraction of glucose utilized came from mucosal and serosal fluids, respectively. This is the consequence of differential volumes of mucosal (15 ml) and serosal fluids (10 ml). Certain mammalian in vitro studies (Wilson, 1956; Newey et al., 1959) have shown that the proportion of exogenous glucose in mucosal and serosal fluids which contributes to tissue metabolism is approximately 70 and 30 per cent, respectively. Hence, the 60/40 ratio used is believed to be a reasonable estimation of an unknown parameter.

Lactic acid content of the media and tissue respiration were determined in an attempt to account for the fraction of glucose utilized. Free glucose, sugar intermediates, and unknown products remaining in the tissue at the end of perfusion are necessarily included in the fraction utilized.

Wilson and Wiseman (1954a, b), Wilson (1954, 1956), and others have noted that a considerable portion of glucose entering rat gut, in vitro, was converted to lactic acid. Lactate can appear in the portal blood and thus is still available for further tissue oxidation. Therefore an increase of lactate in serosal solutions must be recognized as a fraction of total glucose transport. An underestimate of this product is probable since tissue levels were not determined. Concentration of lactate in mucosal and serosal solutions depends on transport mechanisms, differential permeability, and/or both.

Glucose loss via oxidative pathways was calculated from the mean QO_2 values for the initial 4 hours incubation in tissue respiration studies (figure 16). Quantity of glucose oxidized was calculated for comparable tissues, temperatures, and substrate concentration (at 24^{o}C) used in perfusion studies. Calculations were based on an RQ of 1.0 because this would represent the maximum quantity of glucose that could be lost by oxidation.

RESULTS AND DISCUSSION

For accurate comparison glucose absorption and transport rates, obtained in different investigations, must be expressed in terms of tissue mass for a prescribed set of experimental conditions, i.e., general method of study, temperature, substrate concentration, diet, portion of intestine used, etc. Even if all these factors have been accounted for or controlled, the comparison can not be regarded as absolute.

Table II summarizes the average rates of glucose absorption, utilization, and calculated active transport occurring in trout intestine under the influence of several rate controlling factors. The highest absorption rate of 139 μg glucose/mg dry weight/hour was observed in a single mid-gut section at the highest media temperature and substrate concentration employed (Experiment 3). Fisher and Parsons (1949b, 1953), using a recirculation method in isolated rat intestine, found absorption two or three times higher at $37^{\circ}C$ with an initial mucosal and serosal media glucose concentration of 500 mg/100 ml. With reduction of temperature and available substrate to equal the conditions of the trout study the absorption rates would appear to approximate those of trout mid-gut.

TABLE

MEAN RATES OF GLUCOSE ABSORPTION, UTILIZATION, AND CALCULATED
VARYING NUTRITIONAL STATES, TEMPERATURES,

Exp.	Nutr.[1]	T. °C	Init. Med. Gluc. Mg%	N[2] M-G	N[2] C	Glucose Absorbed[3] Mid-gut	Glucose Absorbed[3] Caeca
1	U	14	50	5	3	8.72(.59)	3.94(.15)
2	S	24	50	2	2	7.72(2.99)	0.89(.31)
	FA	24	50	4	3	16.41(1.4)	2.43(.89)
	FB	24	50	4	4	44.74(4.47)	9.04(1.34)
Mean of Exp. 2				10	9	26.00(5.50)	5.02(4.50)
3	FB	24	100	1	1	138.65	24.07
4	FA	14	100	6	6	5.27(.94)	4.29(1.51)

[1]Nutritional status: U = unknown; S = fasted; FA = feeding, food in upper tract; FB = feeding, food only in lower tract.

[2]Number of samples.

[3]Micrograms glucose/mg dry weight/hr ± SE.

[4]Transport = absorption - 3/5 utilization.

II

ACTIVE TRANSPORT IN TROUT MID-GUT AND CAECA SEGMENTS DURING AND SUBSTRATE CONCENTRATIONS

Calculated Glucose Transported[3,4]		Percent of Absorbed Transported		Glucose Utilized[3]	
Mid-gut	Caeca	Mid-gut	Caeca	Mid-gut	Caeca
2.97(.96)	1.00(.13)	30.9	25.4	9.59(1.92)	4.90(.40)
1.68(.10)	0.30	26.2	31.3	10.06(5.18)	0.98(.21)
4.28(.24)	0.13(.09)	26.4	3.7	21.83(3.46)	3.86(1.33)
16.67(2.28)	2.64(.71)	36.9	27.7	46.80(3.79)	10.67(1.22)
8.71(2.34)	1.30(.52)	30.5	20.4	29.46(7.07)	6.25(1.57)
50.82	6.26	26.7	26.0	146.45	29.69
1.77(.31)	0.49(.30)	34.7	9.2	5.64(1.18)	4.41(1.11)

Fisher and Parsons (1953) also found that the rate of glucose movement into serosal media averaged 54 μg/mg dry weight/hour. Wilson and Wiseman (1954a) show similar rates of serosal transfer with everted sacs from rats. A summary (Wilson, 1956) of serosal transfer rates for everted sacs from six species of mammals shows that rates are similar for rat, mouse, guinea pig, and rabbit intestine but two or three times greater in hamster gut. In trout gut segments serosal transfer was negligible. The calculated active transport represents the hypothetical amount of glucose which should appear in serosal fluid if tissue utilization from that compartment did not occur. This derived figure represented about 30 per cent of the glucose absorbed by trout gut. In mammalian studies, serosal appearance of glucose accounted for a similar fraction of total glucose absorbed.

Only two studies dealing with poikilothermic species contain sufficient information for comparison. Musacchia (1962), using everted sacs from catfish, found glucose absorption and serosal transport rates of 7.75 and 3.05 μg/mg dry weight/hour, respectively, at 24°C and 10 mg/100 ml initial glucose. Table II shows that the mean absorption rate of trout mid-gut at the same temperature was almost

3.5 times greater in this study due to the higher sugar content, 50 mg/100 ml. The rate of glucose transport by shark mucosa (Carlisky and Huang, 1962) was approximately the same as calculated mid-gut transport for trout. However, in view of reported leaks in the shark mucosa, little consideration can be given to the data. Whole gut preparations from the shark did not appreciably move glucose to serosal media.

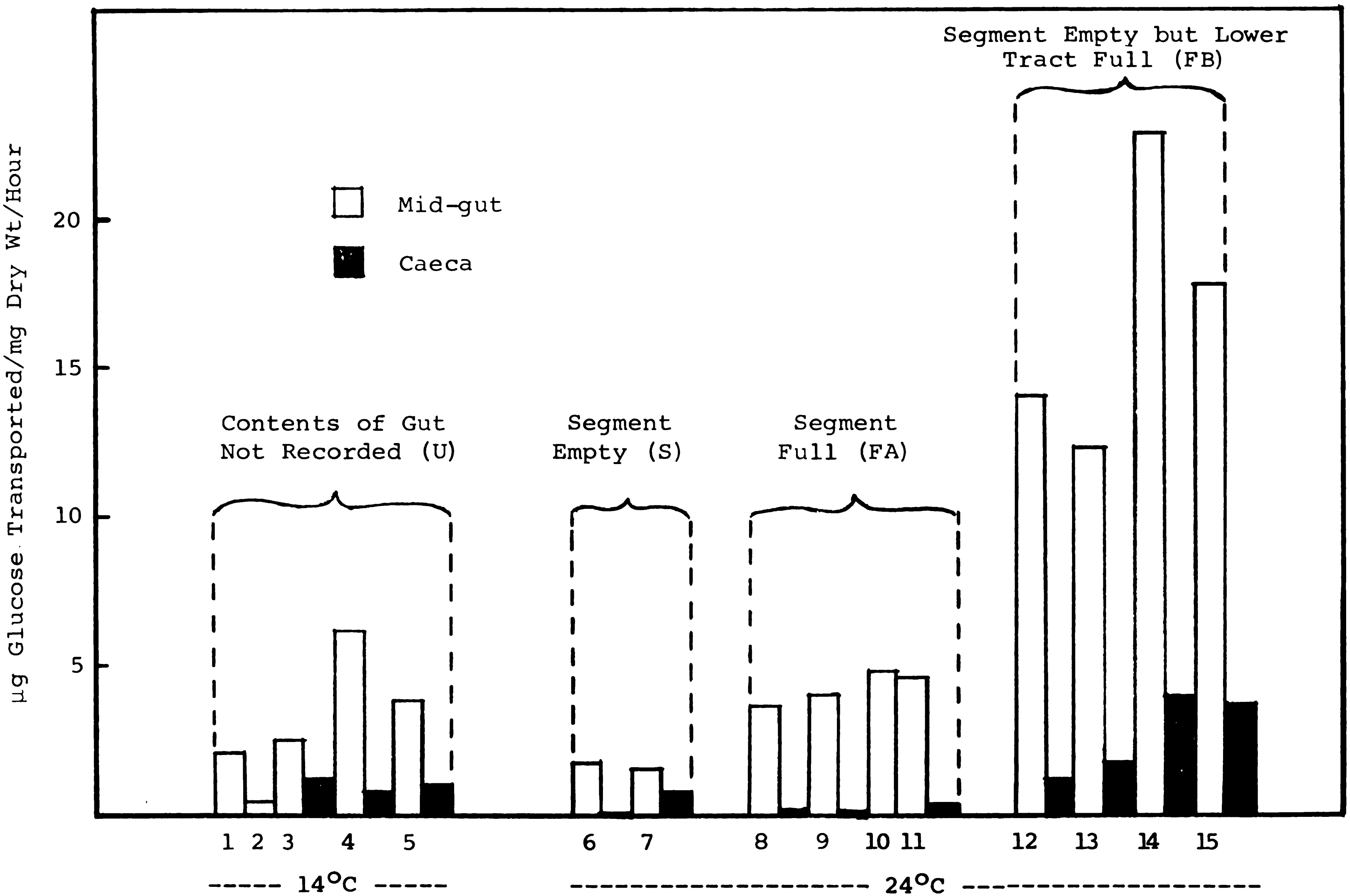
Effects of Nutrition

Studies at 24°C (figure 12) illustrate the influence on calculated active transport of feeding activity and/or presence of food in gut segments prior to perfusion. The series of members (1-15) on the abcissa gives the chronological order in which experiments were run. Only one study was conducted daily and the interval between each was as much as 3 days.

Gut segments from feeding trout (groups FA and FB) exhibited higher transport rates than segments from non-feeding animals (group S). Moreover, caeca and mid-gut sections from intestinal tracts that contained residue in the rectum only (group FB) transferred glucose at a higher rate than filled segments (group FA). Table II illustrates a

Figure 12

Influence of Media Temperature and Feeding Activity on Active Transport of Glucose Across Caecal and Mid-gut Segments with 50 mg Glucose/100 ml in Media (Active Transport = Mucosal Absorption - 3/5 Tissue Utilization; Each Bar Represents an Individual Segment; Abscissa Refers to Sequence of Experiments and Temperature)


Segment Empty but Lower Tract Full (FB)
Mid-gut
Caeca
Contents of Gut Not Recorded (U)
Segment Empty (S)
Segment Full (FA)
µg Glucose Transported/mg Dry Wt/Hour
20
15
10
5
1 2 3 4 5
6 7
8 9 10 11
12 13 14 15
14°C
24°C

similar augmentation of glucose absorption and utilization in feeding trout.

Similar dietary effects have been observed in _in vivo_ studies. For example, Magee (1945) found reduced glucose absorption in the starved rat, cat, and fowl; Marrazzi (1940) indicated appetite loss and decreased food intake was responsible for decreased absorption in adrenalectomized rats. Semi-starvation has an effect which is opposite to that of fasting. Kershaw et al. (1960), using both _in vitro_ and _in vivo_ methods, demonstrated an increased absorptive capacity in guts from rats on reduced food intake compared to individuals fed _ad libitum_. The enhanced transport in group FB trout may reflect a similar semi-starved state whereby emptying of a particular region leaves it in a hyperabsorptive state. However, a more reasonable explanation is that prolonged intermittent feeding progressively stimulated transport processes, because both groups FA and FB were fed in the same manner, and group FB exhibited feeding activity for a longer period of time.

Whether or not the trout guts used in the 14°C studies (figure 12) contained food material was not recorded. Nevertheless, trout in the general holding tanks appeared to be feeding when used. In later experiments (Table II, Exp. 4) at the same temperatures, using well fed animals, glucose transport tended to be lower than in the unknown group although statistical significance could not be demonstrated. This gives good indirect evidence that intestines from trout used in the initial 14°C studies were comparable to groups FA and FB at 24°C.

The exact cause of the effect of diet on sugar absorption is still unknown. Considerable evidence, summarized by Crane (1960), points to nutritional stimulation of epithelial cell proliferation in the crypts between the villi, thus increasing number and decreasing age of cells. Intestinal tissue from non-feeding trout weighed less per unit length than tissue from feeders (Table V). Lodja and Fabry (1959) have demonstrated histochemically increased alkaline phosphatase and esterase in rat small intestine during semi-starvation.

Temperature

The influence of temperature (figure 12) on rate of glucose transport is somewhat obscured by irregular feeding habits. In spite of this variability, gut segments at 24°C had higher mean glucose absorption and transport rates than those at 14°C (Table II). Calculated temperature coefficients

(Q_{10}), using all observations at both temperatures were 2.93 and 1.30 for mid-gut and caeca sections, respectively. If we regard gut segments perfused at 14°C to be from semi-starved animals and these are compared with segments from animals having only the lower tract full, very high Q_{10} values of 5.61 and 2.64 are recorded for mid-gut and caeca, respectively. A true Q_{10} most likely lies between these extremes. Rate of absorption and utilization show similar relationships to temperature.

Cordier and Worbe (1954a) and Csaky and Fernald (1960) show increased absorption of glucose and 3-methyl-glucose in frogs with increasing temperature using <u>in vivo</u> sac preparations. Initial substrate concentrations were about the same in both studies and were above that which produces saturation of the transport system (Csaky and Fernald, 1960). Q_{10} values (10-20°C) in both studies were approximately 2.0. Carlisky and Huang (1962) state that glucose transport by isolated shark mucosa increased 4 to 4.5 fold from 26 to 36°C at an initial glucose concentration well above that which would establish a maximal rate. However, recalculation of their data indicates a Q_{10} closer to 9. At 16°C they found no transport.

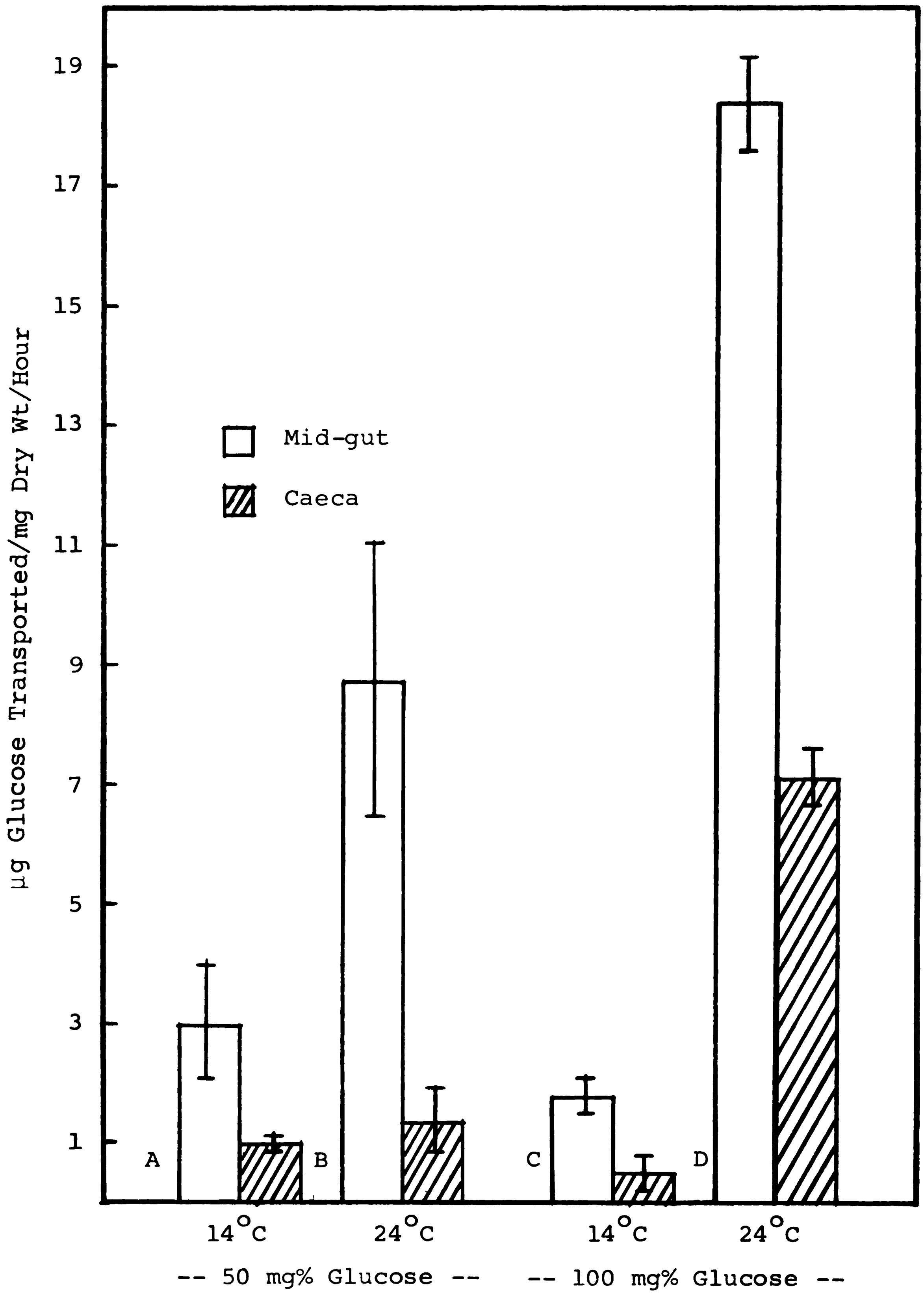
The magnitude of Q_{10} values obtained in studies of glucose transport in poikilothermic intestine suggests a process other than passive diffusion. Moreover, the Q_{10} values of mid-gut are rather high in terms of thermobiological reactions. Since Q_{10} is not a linear function and increases at low temperatures, the high mid-gut Q_{10} values may result from calculations using a base temperature (14°C) which is so low that the rates of enzyme controlled reactions approach those of passive processes. If thermochemical reactions were operating maximally at this reduced state, an increase in substrate concentration would not affect reaction rates. In trout gut (figure 13) increased glucose in the media did not affect glucose transport at 14°C whereas at 24°C large influences were noted. Therefore, by calculating Q_{10} using 14°C as the lower temperature limit in the case of trout gut one would expect Q_{10} to increase directly with substrate concentration.

Substrate Concentration

At 24°C (figure 13) the elevation of glucose in the media from 50 to 100 mg/ 100 ml resulted in a proportionate rise in rate of active glucose transport both in caeca and mid-gut. No influence of substrate concentration was evident at 14°C.

Figure 13

Effect of Media Glucose Concentration on Active Glucose Transport in Caecal and Mid-gut Segments at 14 and 24^{o}C (Brackets Represent 2 SE Units)



µg Glucose Transported/mg Dry Wt/Hour
19
17
15
13
11
9
7
5
3
1
Mid-gut
Caeca
A
B
C
D
14°C
24°C
14°C
24°C
-- 50 mg% Glucose --
-- 100 mg% Glucose --

All trout in group D had been fasted for one week prior to experimentation, whereas in group B most of the animals were feeding. In view of the previous illustration of dietary stimulation of glucose transport in feeding fish, the effect of increased media glucose on transport is underestimated.

The initial glucose content in these experiments is believed to be far below the concentration necessary to saturate a carrier-mediated transport process. Csaky and Fernald (1960) found that glucose absorption reached a maximum rate at about 3 to 4 grams/ 100 ml in frog gut, *in vitro*.

Location

Glucose transport (Table I, figure 4) in mid-gut was 65 to 85 per cent higher than in caeca segments from the same animal. Mid-gut glucose transport also exceeded that of lower gut to about the same degree (Table III). Corresponding absorption and utilization rates were also greater in mid-gut than other regions (Table II). *In vitro* mammalian studies (Fisher and Parsons, 1953; Crane and Mandelstam, 1960) show greater glucose transport per weight of tissue in lower jejunum than in higher or lower segments. Musacchia (1962) found highest transport rates in the most anterior

TABLE III

GLUCOSE TRANSPORT IN TROUT MID-GUT AND LARGE GUT SEGMENTS TAKEN FROM THE SAME ANIMAL

Values recorded as μg glucose/mg dry weight/ hour (temp. = 24°C; media glucose concentration = 100 mg%)

Exp	Segment	Glucose Absorbed	Glucose Transported	Glucose Utilized
1	Mid-gut	41.50	9.10	54.02
	Large gut	16.08	0.95	25.20
2	Mid-gut	51.67	12.14	65.92
	Large gut	18.14	6.23	19.92

portion of catfish intestine whereas the scup, a marine species, exhibited approximately the same rate in all gut regions studied. A caecal region is present in the scup; however, it was not studied.

Both surface area and weight per unit length of trout intestine necessarily increase in the caeca area due to outpocketing of the main tract. Therefore, glucose transport, previously expressed in terms of tissue weight, will give an inaccurate estimate of true regional variation unless the relationship between mucosal surface area and weight is similar in caeca and mid-gut segments. For absolute quantification, it is recognized that transport must be based on surface area.

Surface area measurements (Table IV) show that posterior portions of pyloric caeca expose approximately twice as much mucosal surface area as mid-gut area per cm serosal length. Corresponding values for weight/length relationships indicate that caeca regions have about 2.4 times the mass per unit length as mid-gut. Removal of extraneous tissue (blood vessels, mesentaries, pancreatic and adipose tissue, etc.) from the caeca should reduce this factor to about two. Expression of data in terms of dry weight appears to be as sound as using units of surface area for purposes of comparing transport rates and other metabolic parameters between these regions of the gut tract.

Histological observations of a sphincter-like circular muscle layer at the base of each caecum (figure 3) suggests the possibility that materials may not move into individual caeca during perfusion. This would invalidate previous estimations of caecal glucose transport on a weight basis. However, X-rays of caeca segments perfused with a sodium iodide contrast media (figure 14) show unequivocally that solutions move into individual caeca. The clearing of contents from caecal pouches during perfusion was also observed.

TABLE IV

RELATIONSHIP BETWEEN WEIGHT AND SURFACE AREA PER CM OF TROUT INTESTINE

	Mean Surface Area[1] (cm^2/cm serosal length)	Mean Tissue Weight (mg wet wt/cm s.l.)
Mid-gut	28.22	145 (N = 26)
Pyloric Caeca	58.27[2]	350 (N = 15)
P. Caeca/Mid-gut	2.06	2.41

[1]See Appendix A

[2]Total surface area of caeca and caeca tract included in 1 cm serosal length of caeca tract.

TABLE V

RELATIONSHIP BETWEEN WEIGHT AND LENGTH OF GUT IN FEEDING AND NON-FEEDING TROUT

	mg wet weight/cm serosal length (Mean ± SE)			
	N	Non-Feeding	N	Feeding
Mid-gut	12	139±10	10	168±16
Caeca	9	320±19	5	407±34
Caeca/Mid-gut		2.30		2.42

Figure 14

X-ray of Pyloric Caeca Region

Perfused with (A) NaI

Constrast Medium and

(B) Saline


A
B

Glycogen

Figure 15 shows the anterior to posterior increment of intestinal glycogen along the tract. Within each individual this gradient was consistent. Comparison of regions by matched observation T test indicated mid-gut glycogen concentration was significantly different from caeca and lower gut portions at the 10 and 5 per cent levels, respectively.

Wide standard error brackets resulted from variation in total intestinal glycogen between animals. This variation can be partially accounted for by dietary influences observed in other studies. For instance, pyloric caeca from fasted and feeding trout contained a mean concentration of 6.47 and 9.16 mg glycogen/gm dry weight, respectively. A mean glycogen concentration of 8.3 mg/gm tissue for turtle gut (Fox, 1961) compares favorably with the trout data.

Representative values for glycogen depletion in trout gut segments during 4 hours perfusion are summarized in Table VI. No significant difference between glycogen reduction at various temperatures, glucose concentration and nutritional states could be ascertained. There appears to be a tendency for a higher rate of glycogenolysis in the mid-gut and in segments from previously feeding animals. Increased glycogenolysis in these tissues may result directly from a relatively higher initial glycogen store.

Figure 15

Mean Glycogen Concentration
of Trout Intestine (Brackets
Represent SE; N = 6 Fish)

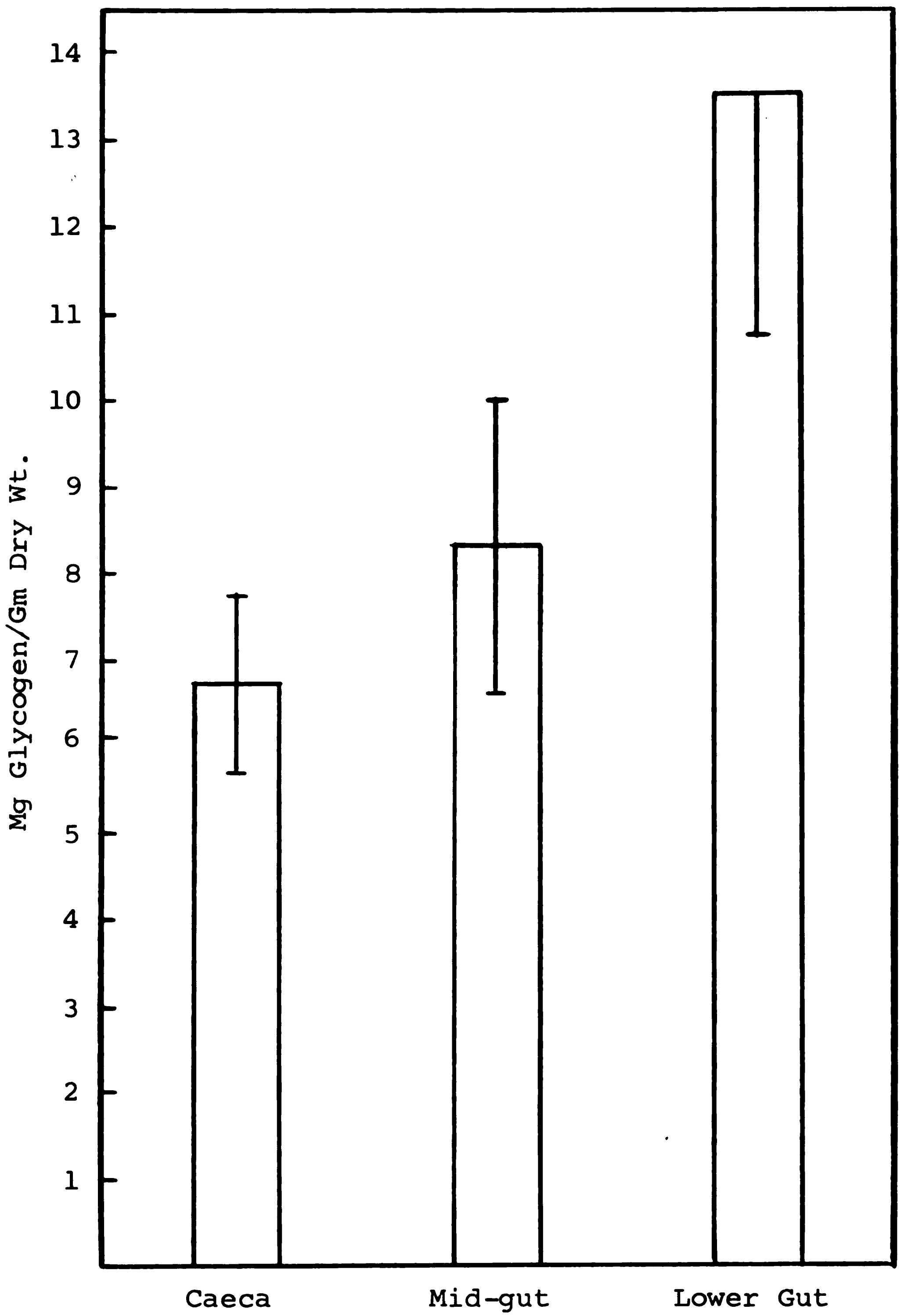

Mg Glycogen/Gm Dry Wt.
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Caeca
Mid-gut
Lower Gut

TABLE VI

RELATIONSHIP BETWEEN GLYCOGEN DEPLETION AND CALCULATED ACTIVE TRANSPORT OF GLUCOSE

Values given as μg/mg dry weight/hour (Mean±SE)

Exp.	Initial Glucose Concentration Mg %	Temp. °C	Nutritional State	Glycogen Depletion		Glucose Transport		% of Transport Accounted for by Glycogen	
				Mid-gut	Caeca	Mid-gut	Caeca	Mid-gut	Caeca
1	50	24	Fasted	0.27±.18	.18±.15	1.68	.30	16	60
			Feeding	1.00±.28	.62±.19	10.47	1.56	10	12
2	100	24	Fasted	0.92±.53	.48±.48	12.40	4.2	7	11
3	100	14	Feeding	.32±.56	.62±.37	1.77	.49	18	76

Glycogen depletion may add to the tissue glucose pool, some glucose which may constitute a portion of the active transport product. Table VI indicates that glycogenolysis can provide a considerable portion of the calculated glucose transported in gut sections either incubated at low temperatures or from starved animals, particularly in caecal sections where glucose transport rate is low. In mid-gut and caecal sections under experimental conditions which stimulate glucose transport, glycogen contribution becomes less important (less than 10 per cent of calculated transport).

Endogenous Glucose Production

Table VII illustrates the rate of appearance of free glucose in media surrounding trout gut segments perfused for 4 hours at 14^{o}C with media containing no glucose. Simultaneous determinations of tissue glycogen gave no indication of a correlation between glycogenolysis and glucose appearance rates. Tissue glucose should be measured for an accurate analysis of this relationship. Significantly greater quantities of free glucose were found in the mid-gut than in the caeca perfusion media. The comparatively low rate of glucose appearance in caecal media supports earlier indications (Table VI) of relatively low glycolysis in this tissue.

TABLE VII

APPEARANCE OF GLUCOSE IN MEDIA AFTER 4 HOURS PERFUSION OF TROUT MID-GUT AND CAECA AT 14°C WITH NO INITIAL GLUCOSE IN MEDIA

Values recorded in µg glucose/mg dry weight/hour

Experiment	Rate of Glucose Appearance in Media		Rate of Appearance in Mucosal and Serosal Fluids			
			Mid-gut		Caeca	
	Mid-gut	Caeca	Mucosal	Serosal	Mucosal	Serosal
1	3.11	0.53	1.10	2.01	0.21	0.32
2	0.88	0.27	0	0.88	0.11	0.16
3	2.00	0.21	1.20	0.80	0	0.21
4	0.67	0.19	0	0.67	0	0.19
Mean	1.67	0.30	0.57	1.09	0.08	0.22

However, it is also possible that low glucose in caecal media was the result of relatively low initial glycogen stores, metabolism, membrane permeability, or their combined influences.

Data in Table VII also indicate that glucose from an endogenous source selectively enters the serosal medium. Fox (1961), using glucose-C^{14} to measure glucose transport in turtle intestine *in vitro*, noted that reduction of the specific activity by dilution occurred in serosal solutions concordant with tissue glycogen depletion. Preferential appearance of endogenous glucose in serosal fluids has been observed by Musacchia (1960), using everted sac preparations of fish gut.

Lactic Acid

Mean rates of lactic acid formation in perfused trout gut segments and its distribution in mucosal and serosal media are summarized in Table VIII. Variable feeding activity of animals in Experiment 1, unlike its effect on glucose transport (Table II), did not appear to influence lactic acid production by mid-gut or caeca. Lactic acid formation increased with both temperature increase (compare Experiments 2 and 3) and higher glucose concentration in the media (Experiments 3 and 4). No significant difference in total

TABLE VIII

TOTAL LACTIC ACID PRODUCTION OF PERFUSED TROUT MID-GUT AND CAECA SEGMENTS AND RESULTING DISTRIBUTION IN THE MEDIA

Exp.	Exp. Cond.	Nutritional Status	Mean Lactic Acid Produced in µg/mg Dry Weight/Hour		Percent of Total Lactate on Serosal Side	
			Mid-gut	Caeca	Mid-gut	Caeca
1	24°C-50 mg% glucose	Fasted	1.76 (1)[3]	1.83 (1)	57	67
		Feeding A[1]	2.55±.46 (4)	2.00±.55 (3)	59±4	77±15
		Feeding B[2]	1.75±.15 (4)	1.88±.21 (4)	88±7	90±3
2	24°C-100 mg% glucose	Fasted	4.96±1.84 (6)	1.62±.26 (6)	73±9	95±5
3	14°C-100 mg% glucose	Feeding	0.87±.19 (6)	1.23±.21 (6)	85±6	92±3
4	14°C-No glucose	Fasted	0.49±.20 (4)	0.84±.19 (4)	94±4	97±2

[1] material initially in segements to be perfused

[2] material in lower tract only

[3] number of segments

lactate formation was demonstrable between mid-gut and caecal regions of the trout intestine.

Wilson (1956) has shown that everted rat jejunal sacs, incubated at 37°C, formed lactic acid at a rate four to five times higher than trout gut. His data apparently represent an *in vitro* rate of aerobic glycolysis since the ratio of oxidative respiration (CO_2 production) to lactic acid formation remains constant with increased temperature (Wilson and Wiseman, 1954a). If oxygen had been the limiting factor at higher temperatures, the ratio would necessarily decrease due to anaerobic glycolysis. Wilson and Wiseman (1954b) have also found highest lactate production in mid-jejunal segments from rat and hamster.

In vitro studies of Wilson and Wiseman have shown that a considerable portion of absorbed glucose may be converted to lactate before reaching the portal blood. However, the *in vivo* studies of Kiyasu et al. (1956) on rats and Atkinson et al. (1957) using dogs, show that glucose in these animals appears in the blood predominantly as free glucose and relatively little lactate is found.

It would be anomalous if relatively large amounts of lactic acid were not formed in gut segments studied *in vitro*, since the absence of a blood supply allows accumulation of all metabolites within the tissue. Although hindered oxygenation of core tissue seems likely, no Pasteur effect was shown in *in vitro* studies at high temperatures (Wilson and Wiseman, 1954a).

During the perfusion of trout tissues, lactate accumulated preferentially in the serosal solution, as is shown in Table VIII. The existence of a similar lactate gradient in rat and hamster intestine has been demonstrated by the everted sac studies of Wilson (1954, 1956). Newey et al. (1955), using rat intestine *in vitro*, found that lactate disappeared from both mucosal and serosal bathing media. This phenomenon was probably due to insufficient substrate in the media, since Wilson (1954) had found that the lactate concentration actually increased in mucosal and serosal fluids during incubation of everted rat intestinal segments when both media initially contained lactic acid and glucose. In neither instance was active lactate transport demonstrated.

The preferential accumulation of lactate on the serosal side of the gut seems best explained on the basis of its endogenous production and subsequent unequal distribution to extracellular fluids through differentially permeable membranes by passive diffusion. Leaf (1959) has shown greater movement of lactate to the serosal side of incubated toad bladder from a twelve-fold greater concentration in the wall. Although similar evidence for the occurrence of this phenomenon in small intestine is lacking, the data obtained from these trout studies definitely supports this hypothesis. Wilson's (1954, 1956) demonstration of a difference in lactate concentration across rat gut may appear to indicate differential permeability. However, his results show that absolute quantity of lactate was generally greater in mucosal solutions because of the much larger mucosal fluid volume.

Differential permeability would also seem to be the most reasonable explanation of glucose movement following intracellular accumulation, resulting from either endogenous production or active transport.

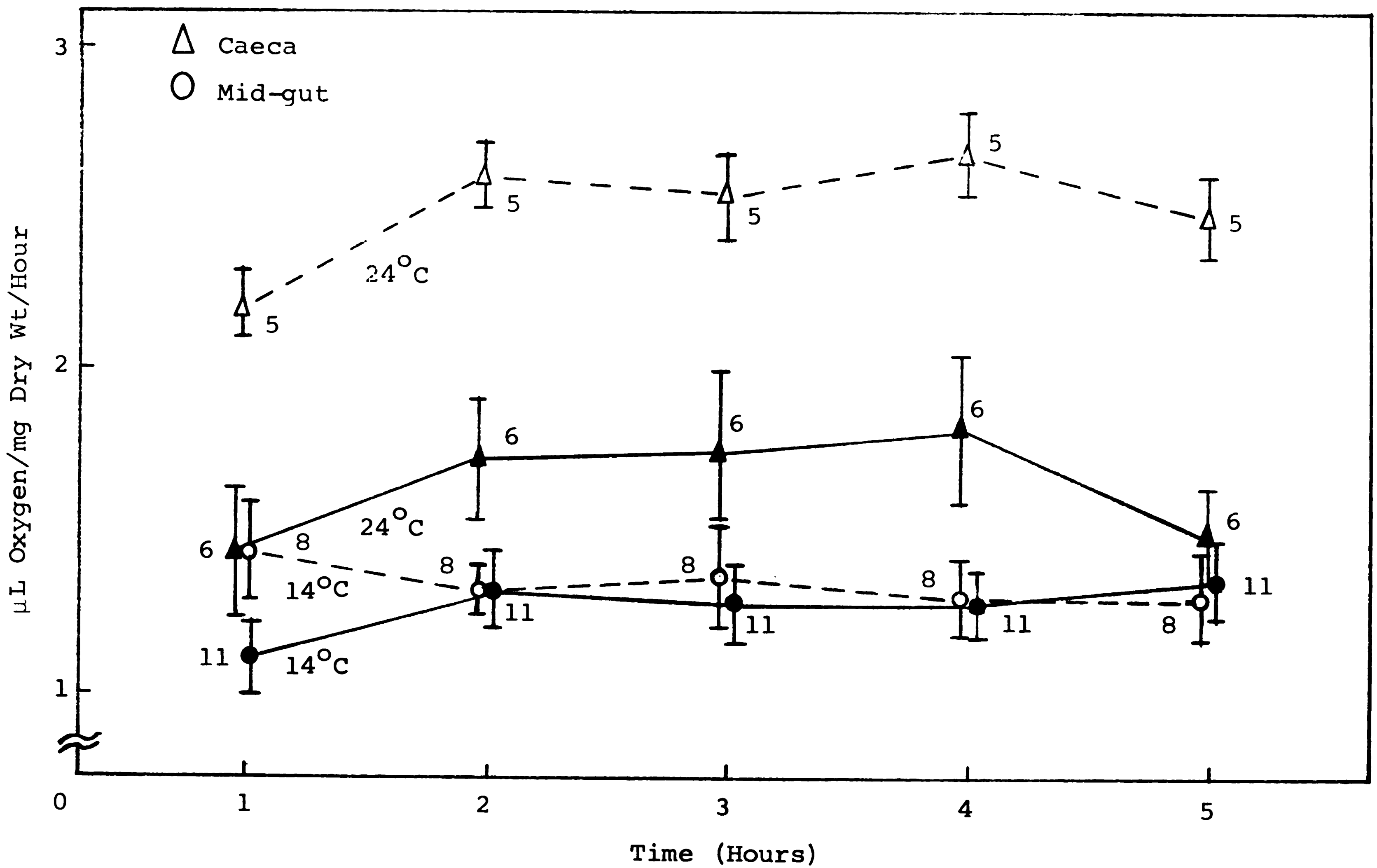
An interesting phenomenon concerning mucosal membrane permeability occurred during perfusion studies with no glucose in the media. It was noted that mucosal fluids exposed to mid-gut and caeca segments contained respectively, 34 and 27 per cent of the glucose entering the entire media and 6 and 3 per cent, respectively, of the total lactate in the media. The relative ease with which glucose enters the mucosal medium is contrary to views on membrane permeability, since glucose is the larger of the two molecules and a mucosal membrane transport mechanism for glucose would be expected to counteract its passive flow into the gut lumen. There is no evidence of active lactate transport. The presence of a transport system apparently has little effect on diffusion of intracellular glucose back into the lumen and the non-specific structure of the mucosal membrane itself exerts primary influence. Perhaps, because of the higher ionization of lactate than glucose, molecular charge of the membrane opposes movement of the former.

Tissue Respiration

The mean hourly Q_{02} values of trout mid-gut and caeca incubated at 14 and 24^{o}C are ploted in figure 16. The wide standard error brackets about the mean obscure attempts to demonstrate consistency of Q_{02} versus time, since the range covered by the brackets results from combined variation of the Q_{02} within individual tissues and between tissues from different animals. An attempt was made to account for individual variation by measuring the deviations of separate observations at hours 1, 3, 4, and 5 from observations at hour 2, which had less initial variability than other periods. The resulting standard error of these differences was at least 50 per cent lower than the original variation.

Figure 16

Q_{O_2} of Trout Mid-gut and Caeca Tissue at 14 and 24^{o}C (Numbers adjacent to curves refer to numbers of samples)



Caeca
Mid-gut
24°C
24°C
14°C
14°C
μL Oxygen/mg Dry Wt/Hour
Time (Hours)

The rate of tissue respiration at 14°C was relatively consistent at about 1.35 μl oxygen/ mg dry weight / hour for 5 hours in both caeca and mid-gut tissue. At 24°C the respiration rate increased to give Q_{02} values of about 1.7 in mid-gut and 2.6 in caeca (between hours 2 and 4). The initial rise between hours 1 and 2 probably resulted from equilibration of tissue respiration to high temperatures, since the tissues had originally been taken from animals kept at 14°C. The fall in respiration after 4 hours is believed to represent autolysis and/ or disintegration of the tissue.

Q_{02} values as high as 23 have been reported for intact duodenal and jejunal segments from rats (Wilson and Wiseman, 1954b) and mice (Dickens and Weil-Malherbe, 1941) incubated at 37°C. These high respiration rates, compared to trout gut, are partially reflected by the higher temperature. However, Q_{10} values of 1.4 and 1.7 for trout mid-gut and caeca respiration, respectively, were determined for the temperature change from 14 to 24°C. If laws of Q_{10} are followed, an additional increase to 34°C would give an even lower Q_{10}, and it does not seem possible that the Q_{02} of trout gut would ever exceed 25 per cent of that of comparable mammalian tissue. Therefore, the differences in intestinal respiration of mammals and trout may reflect true species variation or the poikilothermic nature of the trout. In the trout the Q_{10} for intestinal respiration is much lower than that for transport, absorption, and utilization.

Utilization

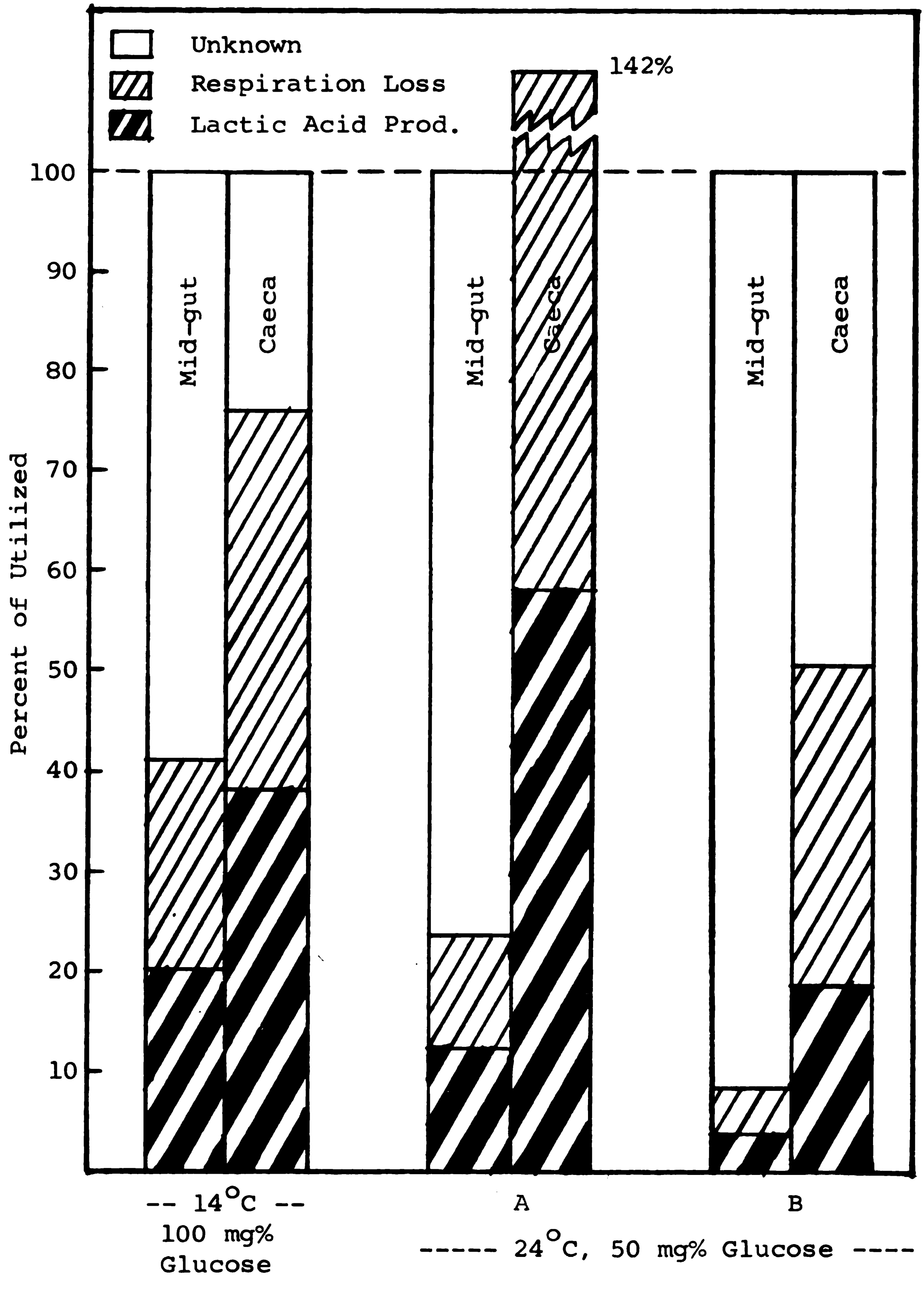
The lactic acid formed in trout gut during perfusion could account for considerable portions of the utilized or disappearing fraction of glucose only in caecal segments incubated at low temperatures and caecal tissue from previously fasted fish or those just beginning to feed (figure 17). In mid-gut sections lactate never accounted for more than 20 per cent of the glucose fraction utilized under the same conditions.

Calculated respiration losses account for about the same proportion of utilization as lactate (figure 17). The ratio of aerobic respiration to lactic acid formation has been found to approximate 1.0 in _in vitro_ studies on mammalian gut (Wilson and Wiseman, 1954a, b; Wilson, 1956).

The combined influences of respiration and lactic acid production never accounted for more than 40 per cent of the glucose utilized even at 14°C, in trout mid-gut, _in vitro_

Figure 17

Per Cent Glucose Utilized Accounted For by
Respiration and Lactic Acid Formation
in Mid-gut and Caeca Segments from
Feeding Trout
(A = gut segments full; B = upper segments
empty, lower tract full)


Unknown
Respiration Loss
Lactic Acid Prod.
142%
100
90
80
70
60
50
40
30
20
10
Percent of Utilized
Mid-gut
Caeca
Mid-gut
Caeca
Mid-gut
Caeca
-- 14°C --
100 mg%
Glucose
A
B
----- 24°C, 50 mg% Glucose ----

(figure 17). In caecal sections, however, the fraction utilized can be largely accounted for in all cases except feeding group B, where glucose transport was observed to be relatively high. In caeca from fed group A, calculated and measured metabolic losses actually exceeded total utilization. This over-correction is not surprising, since oxidation based on an RQ = 1.0 represents the maximum amount of glucose that could be lost by respiration. A more reasonable RQ, resulting from utilization of other nutrients, would be expected. Dickens and Weil-Malherbe (1941) found very low RQ values for rat jejunal mucosa along with high values of lactic acid production and oxidative respiration. No Pasteur effect was evident in their studies. Therefore, lactate appeared to accumulate because entrance into the TCA cycle was inhibited. The maintenance of oxidative respiration indicates that other substrates were utilized, which would account for the low RQ. A similar effect may have occurred in trout caecal tissue, since glycogen storage and glycogenolysis appeared to be low compared to that in mid-gut. Lactic acid production was about the same in both tissues, and Q_{O2} values were comparatively high in caeca.

Metabolism of fats or fatty acids could give rise to a low caecal RQ. Mesenteries covering the pyloric caeca are sites of extensive fat storage and this tissue has long been suspected to be involved in fat absorption. Greene (1912) noted that particulate fat in the epithelium and tunica proporia of pyloric caeca in alantic salmon correlated with presence of emulsified fat in the lumen. The latter was observed along with the presence of considerable bile in the caeca lumen of trout. Rahimullah (1945) has presented qualitative evidence for the presence of lipase in the caeca mucosa of Ophicephalus striatus, a carnivorous fish, but not in the mucosa of a herbivorous form. Schlottke (1939, quoted from Barrington, 1957) found lipase in trout pancreas. Regardless of lipase source, the caeca empirically appear to have a major role in fat digestion and absorption.

General Discussion

As the rate of intestinal glucose absorption increases, carbohydrate metabolism accounts for less and less of the fraction utilized; therefore, proportionally enlarging the unknown fraction. The effect is more pronounced in mid-gut regions but also occurs in the caeca. This relationship leads one to suspect that free glucose in the tissue is at least a partial component of the missing fraction. Fisher and Parsons (1949a) found approximately ten per cent of the glucose absorbed was retained by rat gut. Crane and Mandelstam (1960), in *in vitro* intestine accumulation studies, have shown that the concentration of glucose in tissue fluids is often 200 times that of the media.

The unknown portion may also partially represent transformation of glucose into some other substance. In mammalian gut, *in vivo*, almost all the absorbed glucose reaching the blood is in the form of free glucose and only minor amounts are converted to lactic acid and other products, such as L-alanine (Kiyasu et al., 1956; Atkinson et al., 1957). Nevertheless, these observations do not rule out the possibility of a substantial conversion of transported glucose to amino acids, fatty acids, or other unknown materials in a primitive vertebrate, such as the trout. Also *in vitro* studies are conducive to tissue metabolite accumulation because of the lack of circulation.

If there is little or no conversion of glucose to other products during absorption, glucose accumulates in the tissue against a concentration difference and active glucose transport essentially equals glucose absorption minus carbohydrate metabolism losses. With this interpretation for trout gut, active transport reported herein is underestimated by the fraction of glucose remaining in the tissue.

A luminal membrane transport system tends to concentrate glucose in the mucosa. Upon accumulation, it diffuses passively with the concentration gradient preferentially into the serosal medium, in vitro, or extrapolating to an in vivo situation, into the capillaries, owing to the relative impermeability of the apical epithelial membranes. Evidence that glucose appeared more readily in serosal fluids when the perfusion media contained no glucose, indicates that the presence in the serosal media of at least 50 mg glucose/ 100 ml passively opposes diffusion from the tissue - even during augmented mucosal absorption. The preferential distribution of lactate to the serosal media also appears to be a result of intracellular accummulation, and luminal membrane impermeability.

If there had been appreciable conversion of glucose to unknown products, the intercellular glucose might be maintained at such a low level that luminal glucose would enter the tissue, either by passive or facilitated diffusion. Although this avenue of entrance can not be ruled out for trout gut, it would be extremely contrary to present concepts of active sugar transport.

Although the capacity of the intestine to absorb glucose in trout is comparable to that in mammals at higher temperatures, such temperatures, at least in excess of 24^{o}C are generally lethal to the animal. At lower, more favorable environmental temperatures (14^{o}C), the rate of glucose absorption is relatively slow and it is doubtful that an active transport mechanism is functionally important, even if it exists.

From the viewpoint of nutrition in the trout, even the limited absorption capacity at 14^{o}C probably exceeds requirements, since trout appear to have a relatively inefficient system for handling large quantities of carbohydrates. Phillips et al. (1948) has shown that the fluctuations in blood glucose levels in brook trout, which were force-fed large quantities of sugar, resembled those of a diabetic human being and that hyperglycemic levels of glucose could be rapidly lowered by insulin injections. All evidence indicates that the diabetogenic response was due to lack of insulin, not insensitivity, and is supported by an earlier observation (Hess, 1935) that relatively less islet tissue is present in trout pancreas compared to mammals. Phillips et al. (1948) also found excessive liver glycogen deposition along with a lack of excretion of excess sugar in fish fed high carbohydrate diets. It seems that trout have an ample capacity to absorb and retain glucose, but can utilize only a limited amount due to the low energy requirement imposed by the poikilothermic nature of the species and the cold water environment it inhabits. Furthermore, little carbohydrate is normally included in the diet of this carnivore in nature.

Influence of Chromium on Absorption and Metabolism

Data concerning glucose absorption and metabolism by perfused intestine from trout exposed to 2.5 mg/ 1 chromium for 7 days are summarized in Table IX. Mean calculated glucose transport for caeca and mid-gut from chromium-exposed fish was 40 and 32 per cent lower, respectively, than that of controls (statistically significant at the 10 per cent level). The slightly larger reduction of glucose transport in caecal segments supports earlier evidence (Knoll and Fromm, 1960) that this tissue may be directly envolved in chromium excretion. Glucose absorption and utilization were both inhibited to about the same degree by the presence of chromium (significant at the 10 and 5 per cent levels for caeca and mid-gut, respectively). There were also indications that glycogenolysis and lactic acid formation were reduced in chromium-exposed tissues, although statistically significant differences could not be demonstrated.

Table IX

INFLUENCE OF CHROMIUM ON GLUCOSE ABSORPTION AND METABOLISM IN PERFUSED GUT SEGMENTS FROM FASTED TROUT EXPOSED TO 2.5 MG CR/1 FOR 7 DAYS AT 14°C

(Values expressed as Mean μg/mg dry wt./hr. ±SE)

	Mid-gut		Caeca	
	Chromium Exposed	Control	Chromium Exposed	Control
Absorption	35.8±5.3	54.1±8.8	13.2±1.9	19.3±4.7
Utilization	39.0±6.7	59.6±8.4	9.8±1.3	13.0±2.1
Calculated Transport	12.4±2.0	18.4±2.7	4.0±0.8	7.1±1.5
Glycogen Prod.[1]	-0.5±0.8	3.7±2.1	-0.5±0.8	1.9±1.9
Lactic Acid Prod.	2.3±0.9	5.0±1.8	1.5±0.3	1.6±0.3

[1]Negative values refer to glycogen formation

Experiments on tissue accumulation of chromium (Knoll and Fromm, 1960) in trout exposed to the same chromium-containing environment as used in this study, indicate that chromium levels in caeca and mid-gut would be less than 20 mg/l. All available evidence implies that chromium concentrations of this order do not affect oxidative respiration. Hoffert (1962) found that the Q_{O2} of liver slices and erythrocytes from turtle was not altered during incubation in media containing 20 μg Cr/ ml. Fromm and Stokes (1962) demonstrated that <u>in vitro</u> respiration of pyloric caeca, liver, and kidney sections from trout exposed to 1 mg Cr/ l for as much as 39 days was not different from that of control fish.

The failure to demonstrate a significant effect of chromium on oxidative respiration and glycolysis indicates that the reduction of glucose utilization in perfused, chromium-exposed trout gut was most likely due to reduction of that portion of total utilization unaccounted for. The supposition that this unknown fraction is predominently glucose, along with the relatively greater influence of chromium on glucose absorption than on other metabolic parameters, suggests that a major effect of chromium is inhibition of glucose entrance into the epithelial cell.

Contrary to the findings of this study on trout, Mertz and Schwarz (1960) have stated that glucose uptake of epididymal fat in rats is stimulated by trivalent chromium. However, glucose movement into fat tissue is not considered to be an active process such as intestinal glucose transport. Since proteins are capable of binding metal ions, it is possible that chromium exerts its influence by binding active sites of proteins possibly involved in active glucose transport. However, unlike other metallic ions, the action of chromium appears to be more specific, and similar to that of phlorizin. This glycoside has been found to reduce drastically glucose absorption from the intestinal lumen at concentrations which will not appreciably alter the rates of energy-yielding reactions (Crane, 1960; Wilson, 1962).

If this hypothesis concerning the action of chromium ion in trout gut is true, chromium pathogenesis may originate with impaired nutrient absorption. Absorption is fundamental to all other nutritional processes and if it fails, other processes fail.

CONCLUSIONS

The experiments on absorption and metabolism of D-glucose by trout gut segments, _in vitro_, have shown that:

1. Mid-gut segments consistently gave higher rates of glucose absorption and transport than caeca and large gut sections on a tissue dry weight basis. The relationship between dry weight and mucosal surface area is constant for caeca and mid-gut regions.

2. Glucose absorption capacity of mid-gut was comparable to that of mammalian small intestine at high media temperatures and substrate concentrations.

3. Glucose absorption is increased greatly by increased media temperature, media glucose concentration, and feeding activity of the fish whereas glycogenolysis, glycolysis, and oxidative respiration were relatively constant under the same conditions in all regions of the gut. Catabolic transformation of absorbed glucose became important only during instances of low glucose absorption.

4. Rates of glucose and glycogen disassimilation were similar in caeca and mid-gut regions.

5. Luminal epithelial membranes are more impermeable to diffusion of endogenously produced glucose and lactic acid than are serosal membranes.

6. Under the experimental conditions used herein, hexavalent chromate ion inhibits glucose absorption in both mid-gut and caeca. No significant effect on other metabolic processes was demonstrated.

7. Assuming that a portion of the unaccounted for fraction of total glucose utilization represents glucose still in the tissue, then one could conclude from the data presented that an active mechanism for glucose transport is present in the trout gut mucosa.

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APPENDIX

Table A

MEASUREMENTS OF LENGTH, CIRCUMFERENCE AND SURFACE AREA FOR PYLORIC CAECA AND MID-GUT REGIONS OF TROUT GUT

	Measurements	N[1]	Mid-gut	N[1]	Caeca Tract	N[1]	Caeca
A	Mean mucosal circumference (mm)	24	50.4	24	51.1	48	17.2
B	Mean serosal circumference (mm)	24	15.5	24	16.7	48	5.3
C	Mucosal c.[2] / Serosal c.	24	3.3±.11	24	3.1±.12	48	3.2±0.8
D	Mucosal length[2] / serosal length	6	5.6±.18	3	5.8±.69	13	1.6±.12
E	Mean caeca length (mm)					14	9.0
F	Mean no. caeca/cm caeca tract[5]					24	13.0
G	Surface area (cm^2/cm serosal length)		28.22[3]		29.64[3]		28.63[4]

NOTE: All lengths and area parameters for caeca and caeca tract were obtained only from that portion of pyloric caeca used in perfusion studies

[1]Number of histological sections

[2]Mean ± SE

[3]G = A x D (in cm)

[4]G = A X D (in mm) X E X F

[5]Mean No. of Caeca/animal = 67 (52 - 92) mm; N = 14

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