OVERDUE FINES ARE 25¢ PER DAY
PER ITEM

Return to book drop to remove
this checkout from your record.

CONTRIBUTION OF HYDROLYTIC PRODUCTS OF FOOD DIGESTION AND BILE TO THE POSTPRANDIAL INTESTINAL HYPEREMIA

By

Peter Ramutis Kvietys

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Physiology

1979

ABSTRACT

CONTRIBUTION OF HYDROLYTIC PRODUCTS
OF FOOD DIGESTION AND BILE TO THE
POSTPRANDIAL INTESTINAL HYPEREMIA

By

PETER RAMUTIS KVIETYS

Blood flow to the small intestine increases after a meal and this hyperemia appears to be localized to that portion of the intestine exposed to chyme. Constituents of chyme responsible for the hyperemia are hydrolytic products of food digestion and addition of bile to the digested food enhances the hyperemic effect. The major aims of the present study were to identify which of the hydrolytic products of carbohydrate, protein, and lipid digestion are responsible for the hyperemia and to determine whether bile can influence the vascular effects of these products. The venous outflows of two in situ jejunal segments were simultaneously measured while various food chemicals at postprandial intrajejunal concentrations were placed into the jejunal lumen, with or without bile. The effect of bile on the absorption of glucose and oleic acid from the jejunum was also studied.

A mixture of glucose (150 mM), 16 amino acids (25.2 mM), and micellar lipids (containing 40 mM oleic acid, 20 mM monoolein, and 10 mM taurocholate) increased local blood flow. A solution containing 16 amino acids did not increase flow until its concentration was increased from 25.2 to 252 mM. The hyperemic effect of the 252 mM mixture of amino acids

could be attributed to glutamic (28 mM) and aspartic (20 mM) acids. The other 14 amino acids (glycine, valine, leucine, isoleucine, threonine, phenylalanine, tyrosine, tryptophan, cystine, methionine, histidine, lysine, arginine and proline) did not increase flow. Glucose alone increased flow, but the hyperemic effect of lipids required the presence of taurocholate. Individually, oleic acid, monoolein, or taurocholate did not increase flow but a combination of taurocholate and either of the two lipids increased flow.

Bile (10 or 33%) in the lumen did not alter jejunal flow. In the presence of 10% bile a 25.2 mM mixture of amino acids, a 37.5 mM mixture of 3 dipeptides, or 20 mM caproic acid did not alter flow, 20 mM oleic acid increased flow, and the hyperemic effect of 200 mM glucose was doubled. In the presence of 33% bile, the amino acids, caproic acid, and oleic acid all increased flow, and the hyperemic effect of glucose was tripled. Triglycerides had to be digested and their lipolytic products solubilized in bile before they produced a hyperemia. The hyperemic effect of fatty acids was related to the chain length and concentration of the fatty acids.

Bile enhanced the hyperemic effects of glucose but did not alter glucose absorption or the increase in oxygen consumption produced by glucose. At pH 7.0 oleic acid produced a hyperemia only in the presence of bile. Oleic acid-1-^{14}C was absorbed whether or not bile was present, but a greater amount was absorbed in the presence of bile. At pH 9.5, oleic acid produced the same hyperemia with or without bile, and again bile enhanced the absorption of oleic acid-1-^{14}C.

In conclusion, of the major hydrolytic products of food digestion, only glucose increases jejunal blood flow in the absence of bile or taurocholate. At both high (33%) and low (10%) concentrations bile enhances the hyperemic effects of glucose and allows the long chain lipolytic products to produce a hyperemia. Amino acids produced a hyperemia only in the presence of 33% bile or at concentrations ten times those found in the lumen postprandially. Thus, it appears that the hydrolytic products of carbohydrates and lipids contribute greatly to the postprandial intestinal hyperemia, whereas, those of protein contribute little. Finally, bile does not alter the vascular effects of glucose and oleic acid through an enhancement of their absorption.

ACKNOWLEDGEMENTS

I am grateful to the members of my advisory committee for their assistance and guidance.

I am especially grateful to Dr. Ching-chung Chou for sharing his expertise in the area of gastrointestinal circulation and Dr. David Reinke for many stimulating discussions on gastrointestinal physiology.

I also wish to thank William Stoffs for his assistance in performing some of the chemical analyses, and my colleagues Robert Gallavan, Siu Po Sit, and Richard Nyhof for their surgical assistance and critical discussions on the data presented herein.

A special thanks is extended to Miss Theresa Solak for her clerical assistance and her patience in dealing with me during the preparations of the drafts for this dissertation.

TABLE OF CONTENTS

LIST OF TABLES

continued

LIST OF TABLES, continued

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Anticipation and ingestion of food elicits a generalized cardiovascular response indicative of an increase in sympathetic nervous activity (18, 41, 42, 103, 104, 105). Within five to fifteen minutes after completion of the meal, the cardiovascular response becomes limited to an increase in flow through the superior mesenteric artery (SMA) which persists for up to five hours (41, 42, 103, 104, 105). The increase in flow through the SMA is due, in part, to a hyperemia in the intestinal vascular bed, which is largely confined to the mucosa of that portion of the intestine exposed to chyme (26). Postprandial chyme contains digested and undigested food, pancreatic enzymes, and bile. Recent studies show that intrajejunal instillation of digested food increases local blood flow while instillation of undigested food, pancreatic enzymes or bile does not (95). Furthermore, addition of bile to digested food markedly enhances the hyperemic effect of food (95). The above findings indicate that the constituents of chyme producing the postprandial intestinal hyperemia may be the hydrolytic products of food digestion and that bile may play an important auxiliary role in the hyperemia by potentiating the vascular effects of these products. There are many hydrolytic products of food digestion in the postprandial gut lumen and which of them are responsible for the hyperemia is still not clear. Nor is it clear whether bile potentiates the vascular effects of all or only some of these hydrolytic products.

The present study was designed to systematically examine the local vascular effects of intrajejunal placement of various hydrolytic products of carbohydrates, protein, and lipid digestion and to determine whether the vascular effects of these substances can be altered by bile. In addition, the possiblity that bile enhances the hyperemic effect of digested food by enhancing the absorption of glucose and fatty acids in the food was examined.

CHAPTER II

REVIEW OF LITERATURE

A postprandial splanchnic hyperemia has been demonstrated in man (15, 84), baboons (105), dogs (18, 41, 42, 56, 103, 104), and rats (89).

Prior to 1965 most of the studies concerned with the cardiovascular response to feeding made use of indirect methods to measure cardiac output and peripheral blood flow in rats, dogs, and man. Cardiac output was measured by gas equilibrium (44, 50), dye dilution (30), BSP (88), or ballistocardiograph (86) methods while blood flows to various organs were measured by BSP clearance (15), plethysmographic (1), thermostromuhr (36, 56), and K^{42} distribution (89) methods. These studies showed that during digestion of a meal there was an increase in blood flow to the splanchnic viscera (15, 56, 89), an increase in cardiac output (30, 44, 50, 56, 86, 88, 89), and either a slight increase (30) or no change (50) in systemic arterial pressure. These results suggested that the enhanced blood flow to the digestive organs was supplied by an increased cardiac output. This concept was further supported by the findings that after meals blood flow increased to the extremities (1, 56, 89), heart (36, 89), kidneys (89), and brain (89) as well as the splanchnic viscera. Thus, prior to 1965, the prevalent concept of postprandial cardiovascular adjustments held that there was an augmentation of cardiac output which was shared by all of the organs of the body.

After 1965 many investigators interested in the cardiovascular response to feeding made use of flowmeters to continuously monitor blood flows through individual arteries of conscious dogs and primates (18, 41, 42, 103, 104, 105). The data generated by this method indicate that the cardiovascular response to feeding can be divided into two distinct phases: 1) those occurring during anticipation and ingestion of food, and 2) those occurring during digestion and absorption of luminal contents. During the anticipation-ingestion phase cardiac output, arterial pressure and heart rate increased. Simultaneously, the resistances of the superior mesenteric and renal arteries increased by 40 and 25% respectively while those of the iliac and coronary arteries decreased by 40 and 62% respectively (104). These changes however are transient, lasting from 5 to 15 minutes after ingestion of a meal (18, 41, 42, 103, 104, 105). By 20 minutes after the meal, cardiac output, arterial pressure, and heart rate returned to control levels and remained there for several hours. Simultaneously, blood flow through the superior mesenteric artery returned to control and then increased to 15 - 200% above control (104). Flows through the renal and coronary arteries returned to control, and flows through the iliac artery decreased by 10 - 25% (41, 42, 103, 104, 105). These more recent studies suggest that while anticipation and ingestion of food elicit a generalized cardiovascular response, during the digestive period there is a redistribution of an unchanged cardiac output to the digestive organs at the expense of blood flow to the extremities.

Although the blood flow to most of the small intestine is supplied by the superior mesenteric artery, only the vascular beds of select segments of the intestine appear to participate in the postprandial mesenteric

hyperemia (26, 70). Chou et al. (26) have shown that in anesthetized dogs, infusion of digested food into the duodenal lumen increases SMA flow but does not affect the flow through an isolated jejunal segment which is not exposed to food. Furthermore, placement of digested food into one of two isolated in situ jejunal segments increases flow only to the segment containing food, but does not alter the flow to the other segment containing normal saline (26, 70). The hyperemia in the segment containing food is a result of an increase in flow to the mucosal layer of the intestinal wall; flows through the submucosal and muscular layer are not altered (26). Thus, the postprandial mesenteric hyperemia appears to be localized to the mucosa of that portion of the intestine which is exposed to chyme.

Chyme in the lumen could effect intestinal blood flow through a mechanical stimulation or irritation of the mucosa. Biber et al. (8) have shown that mechanical stimulation of the mucosa of an isolated jejunal segment (by sliding a vinyl tube back and forth intraluminally) produces an increase in local blood flow. A recent study by Sit et al. (95), however, suggests that postprandial chyme does not cause the same degree of mechanical stimulation as that produced in the study by Biber et al. They have shown that luminal placement of digested food can increase jejunal blood flow, but placement of undigested food has no vascular effect. If mechanical stimulation was responsible for the hyperemia both undigested and digested food should have increased flow. In the same study, it was shown that luminal placement of the supernatant of digested food increases flow, but placement of the precipitate does not. Again, if mechanical stimulation of the mucosa was a significant factor in producing the

hyperemia, the precipitate (containing most of the particulate matter) should have increased flow, not the supernatant.

Changes in pH of gut contents after meals may affect intestinal blood flow. Intraduodenal placement of 0.127 N HCl (pH, about 0.9) increases SMA blood flow (38). Perfusion of the duodenum with Tyrode's solutions having a pH of 2.0 or 1.5 increases local blood flow (64). However, duodenal perfusion with Tyrode's solutions having pH values between 2.5 and 11 does not alter local blood flow (25, 64). Postprandially, the lumen contents have pH values ranging from 3.5 to 7.4 (14, 40). Thus the observed vascular effects due to the low luminal pH (2.0 or below) are pharmacological and changes in the pH of lumen contents after meals do not significantly contribute to the postprandial intestinal hyperemia.

The effect of osmolality of lumen contents on intestinal blood flow has also been studied. Intrajejunal placement of a polyethylene glycol (PEG) solution having an osmolality of 3,000 mOsm/kg increased local blood flow by 14% (21). Placement of PEG solutions having osmolalities of 300, 1000, and 1500 mOsm/kg did not affect jejunal flow (21, 70). The lumen contents after a meal have osmolalities ranging from 220 to 320 mOsm/kg (40). Thus, the intestinal hyperemia produced by high luminal osmolality (above 1,500 mOsm/kg) is aphysiological and probably occurs only in pathological states such as the "dumping syndrome". In the normal individual it is doubtful that changes in the osmolality of lumen contents after meals contribute to the postprandial intestinal hyperemia.

Electrolytes are also present in the gut lumen after meals and they may affect local intestinal blood flow. Chen (20) studied the vascular effects of intraileal placement of isosmotic solutions of NaCl (154

mEq/lit.), KCl (160 mEq/lit.), $MgCl_2$ (233 mEq/lit.) and $CaCl_2$ (233 mEq/lit.). Isosmotic solutions of NaCl, $MgCl_2$, or $CaCl_2$ slightly decreased flow, while KCl had a variable effect. Available data show that the concentrations of electrolytes in ileal contents postprandially are as follows: 100 - 150 mEq/lit. for Na^+, 1 - 4 mEq/lit. for Ca^{++}, 4 - 8 mEq/lit. for K^+, and 40 - 110 mEq/lit. for Cl^- (40). Although, the postprandial concentrations of Mg^{++} have not been measured, the average daily diet contains about 20 mEq/lit. of Mg^{++} (38). Thus, it appears that only the decrease in blood flow observed with NaCl in the ileal lumen can be considered physiological. However, recent studies in our laboratory (26, 70) have shown that isosmotic NaCl solutions (154 mEq/lit.) are without vascular effects in the upper small intestine. Perfusion of the duodenum with normal saline (isosmotic NaCl) does not alter flow through the superior mesenteric artery (70). Placement of normal saline in an isolated jejunal segment does not alter the local venous outflow from that segment (26, 70). The effects of luminal placement of $CaCl_2$, $MgCl_2$, or KCl on upper small intestinal flow have not been studied. Information is not available on the postprandial luminal concentrations of other electrolytes (iron, copper, or phosphates) or their vascular effects when placed in the intestinal lumen. However, i.a. infusions of ferric iron, but not ferrous iron, produces vasodilation in SMA vascular bed (24).

The postprandial luminal concentrations of nucleotides and related compounds are not known. Although the vascular effects of luminal placement of these compounds have not been studied, they are vasodilators when infused intra-arterially into the intestinal vascular bed. Intra-arterial infusion of ATP or adenosine into natural or constant flow preparations of

the canine ileum decreased vascular resistance (29). Also, intra-arterial infusion (10μmol/min.) of ATP, AMP, adenosine, c AMP, or dibutyryl c-AMP into the superior mesenteric vascular bed greatly increased flow (74). Thus, these compounds, present in ingested foodstuffs, could contribute to the postprandial intestinal hyperemia, but until their postprandial lumen and blood concentrations are known, no definitive conclusion can be drawn.

Vitamins are also present in ingested foodstuffs, but their postprandial luminal concentrations are not known and their vascular effects have not been studied.

Chyme traversing the small intestine postprandially also contains digested and undigested food, pancreatic enzymes and bile. Food, digested in vitro by a pancreatic enzyme preparation, increased local blood flow when placed in the jejunum of anesthetized dogs (26, 70, 87, 95) and the increase in flow was confined to the mucosal layer (26). Placement of undigested food, however, did not alter jejunal blood flow. Furthermore, the supernatant of digested food increased flow but the precipitate did not alter flow (95). Because the pancreatic enzyme preparation used to digest the food was not vasoactive (70, 95), it appears that the substances responsible for the hyperemia are likely to be the hydrolytic products of food digestion. Bile, another constituent of chyme, did not alter local blood flow when placed in the jejunum (95, 97). However, the addition of this bile to digested food markedly enhanced the hyperemic effect of the food (95).

Most of the information on the postprandial luminal concentrations of various hydrolytic products of food digestion stems from intubation studies in humans (2, 14, 40, 59, 82, 83, 85). The subjects were intubated via the

nose (14, 59, 83) or mouth (2, 40, 85) with flexible tubing containing sampling holes. The level of sampling was determined either by flouroscopy (85) or estimated from the distance the sampling holes of the tubing were from the nose or mouth (2, 14, 59, 82, 83). Intestinal samples were aspirated several times from 15 minutes to 4 hours after the subjects ingested various test meals. In general, there did not appear to be any systematic differences in composition among upper small intestinal samples collected from 30 minutes to 3 hours after the meal (2, 59, 83) and usually the results were either pooled or representative data presented. The results of these studies have an important bearing on the present study and thus have been reviewed in greater detail below.

Borgstrom et al. (14) measured the intrajejunal concentrations of glucose, fat, and protein of subjects fed a 500 ml test meal containing 30 gm of fat, 75 gm of carbohydrate, and 25 gm of protein (with ^{131}I labelled RIHSA). Glucose was determined calorimetrically, non-phospholipid fat was determined gravimetrically after liquid extraction of the intestinal sample, and food protein was estimated from the total ^{131}I activity in the sample. The amount of glucose in the jejunal aspirates ranged from 5 - 40 mg/ml (on a molar basis, 27.8 to 222.2 mM/liter). The concentration of non-phospholipid fat ranged from 1 - 10 mg/ml with about 65 - 70% as free fatty acids. The concentration of food protein was 10 - 20 mg/ml with 50 - 60% as hydrolyzed protein. No attempt was made to analyze the composition of the hydrolyzed protein.

Later, more specific information on the state of lipids in the intestinal lumen postprandially was generated from Borgstrom's laboratory (59). In this study, Hofmann and Borgstrom aspirated duodenal contents

from men and women fed a 400 gm test meal containing lipids in the form of corn oil (tryglycerides). After centrifugation, the samples contained a large clear supernatant (termed the micellar phase) with oil droplets present on the surface. The oil portion never made up more than 5% of the sample. The concentration of triglycerides, diglycerides, monoglycerides, and fatty acids were determined gravimetrically after selective liquid extraction of the various forms of lipids. The lipids of the micellar phase were chiefly fatty acids and monoglycerides with smaller amounts of di- and triglycerides. The lipids of the micellar phase contained significantly more fatty acids and less di- and triglycerides than did the oil phase. The monoglyceride composition of the two phases was not significantly different. The total concentration of the various lipids was as follows: fatty acids ranged from 4.1 - 75.0 mM/liter, monoglycerides ranged from 0.0 - 16.0 mM/liter, diglycerides ranged from 0.0 - 6.0 mM/liter and the triglycerides ranged from 0.0 - 5.6 mM/liter. The authors concluded that their "results are consistent with the hypothesis that intestinal lipid, during fat digestion, is selectively partitioned between a micellar and an oil phase and that absorption of dietary lipid takes place from a micellar solution containing chiefly fatty acid and monoglyceride" (59).

Olmstead et al. (85) measured the free amino acid concentration of the proximal jejunum of subjects fed four different meals: 1) 200 gm of lean beef, 2) 18 g gelatin, 3) three boiled eggs, and 4) 450 ml of whole milk. After deproteinization of the aspirates with picric acid the samples were filtered, and the concentrations of 17 amino acids determined chromatographically with an automatic amino acid analyzer. The greatest intrajejunal concentrations of all 17 amino acids was observed after the

beef meal (17.5 mM/liter) and the lowest after the milk meal (3.9 mM/liter). Intermediate concentrations were observed after the gelatin and egg meals (6.0 and 9.2 mM/liter, respectively).

Nixon and Mawer (83) showed that proteins were not completely hydrolyzed in the intestine to amino acids. They sampled duodenal or jejunal contents of five men and one woman fed a 400 ml milk-protein meal containing 53 gm of lactose, 24 gm of fat, and 15 gm of protein (103 mM mixture of 17 amino acids). The intestinal samples were fractionated by centrifugation and gel filtration and the fractions hydrolyzed with 6N-HCl and the free amino acid concentrations determined with an automatic amino acid analyzer. The results indicated that the greatest percentage (about 41%) of the total amino acids was contained in the fraction containing mostly amino acids and small peptides. In this fraction the total amino acid concentration was 30 mM/liter, with the free amino acid concentration being about 3.8 mM/liter.

Adibi and Mercer (2) also studied the changes in intraluminal concentrations of amino acids in free and peptide form after ingestion of meals. Jejunal samples were aspirated from subjects fed a meal consisting of 120 g of corn starch, 40 gm of olive oil, 5 ml of lemon juice, 4.5 gm of salt, 500 ml of water and 50 gm of bovine serum albumin as the protein. The samples were deproteinized with sulfosalicylic acid and centrifugation. The free amino acid concentration of 17 amino acids was determined with an automatic amino acid analyzer before and after hydrolysis. The concentration of amino acids in peptide form was the difference between the concentrations of the same amino acid before and after hydrolysis. They found that the concentrations of free amino acids totalled

29 mM/liter, postprandially; while the concentration of amino acids in peptide form was about 118 mM/liter.

In summary, the results of these intubation studies indicate that the luminal concentration of various hydrolytic products of food digestion after meals varies widely. The postprandial luminal concentrations of glucose ranges from 28 to 222 mM/liter, that of amino acids from 3.9 to 29 mM/liter, that of monoglycerides from 0 to 16 mM/liter, and that of fatty acids from 4 to 75 mM/liter. If these food chemicals at these concentrations can be shown to increase intestinal blood flow when they are placed in the lumen, this could be considered evidence that they are responsible for the postprandial intestinal hyperemia.

The literature contains several studies in which the vascular effects of intraluminal placement of food chemicals has been examined. Placement of 10 to 15 ml of 139 to 2778 mM glucose solutions into isolated in situ canine jejunal segments (10 to 15 cm in length) increases the local venous outflows from the segments (21, 102, 114). Furthermore, intrajejunal placement of a 2778 mM glucose solution increases the blood flow through the mucosal layer of the jejunal wall; flows through the submucosal and muscular layers are not changed (114). However, in cats, intraduodenal instillation of smaller volumes (0.5 to 4.0 ml) of 278 mM glucose does not alter flow through the SMA (38). Also, when glucose solutions are perfused through the canine duodenum or jejunum, 1389 to 2778 mM glucose increases local blood flow while 278 mM glucose does not (22, 101). Less information is available on the local vascular effects of luminal placement of amino acids. In dogs, placement of an isotonic glycine solution (300 mM) into an isolated in situ jejunal segment increases

the venous outflow from that segment (102). Also, in anesthetized cats, intraduodenal infusion of 127 mM phenylalanine increases SMA flow (38). Unfortunately, both of these studies used concentrations of amino acids which probably do not occur in the lumen postprandially (2, 83, 85). Finally, although there are no reports in the literature on the vascular effects of fatty acids, instillation of 0.5 to 2.0 ml of corn oil into the duodenum of anesthetized cats increases SMA flow (38). The above findings, although incomplete and somewhat controversial, do suggest that the hydrolytic products of food can increase intestinal blood flow when they are placed in the lumen.

Although a postprandial intestinal hyperemia has been well documented, the mechanism(s) by which chyme in the lumen initiates the hyperemia are not, as yet, clearly defined. The available literature on the subject implicates three major mechanisms. They are; 1) activation of a nervous reflex (87, 103, 104), and 2) the release of vasoactive humoral substances from the gut wall into the circulation (38, 63), either directly or indirectly through an increase in intestinal metabolism (17, 42, 102).

The mesenteric vasodilation during digestion appears to involve a cholinergic pathway. Vatner et al. (103, 104) showed that the decrease in SMA resistance during digestion can be abolished or attenuated by cholinergic blockade with atropine, but not by vagotomy or adrenergic blockade with phenoxybenzamine or propranolol. Post et al. (87) showed that, in anesthetized dogs, prior treatment of the mucosa with a local anesthetic, dibucaine, attenuates the local hyperemic response to intrajejunal placement of digested food. The hyperemic effect of luminal placement of 50% glucose is also abolished or attenuated by mucosal

anesthesia (21, 114). These findings indicate that the cholinergic nerves involved in the postprandial hyperemia may be local and possibly those of the enteric intramural nerve plexuses.

The involvement of humoral substances in the postprandial hyperemia was suggested by the results of bioassay and cross-perfusion experiments (38, 63). Intraduodenal infusion of food increases the local venous outflow and decreases the vascular resistance of an isolated bioassay jejunal segment being perfused by the duodenal venous blood (63). Also, in cross-perfusion experiments, intraduodenal instillation of oil in a donor animal increases the donor's SMA flow, as well as the flow through the recipient's SMA being perfused with the donor's aortic blood (38). These data indicate that contact of chyme with the mucosa could cause the release of vasoactive substances into the circulation. Among the possible substances are the gastrointestinal hormones, secretin, gastrin, and cholecystokinin (CCK). These hormones are released into the circulation from the mucosa of the gut after meals to regulate secretory and motor activity of the digestive organs (12, 51, 65, 75). Chou et al. (23) have recently studied the vascular effects of local intraarterial infusion of these three hormones in the duodenum, jejunum, heart, kidney, forelimb, spleen, and skin and muscle of the forelimb of anesthetized dogs. Secretin produced a similar vasodilation in all organs. Pentagastrin produced vasodilation only in the duodenum and jejunum. Unfortunately, to produce their vasodilatory effects, the local concentrations of secretin and gastrin in the arterial blood had to be raised to levels much greater than those occurring after meals. CCK, at concentrations which occur in the circulation postprandially, produced vasodilation in the duodenum and jejunum but did not

affect the other organs studied. CCK has a selective vasodilator effect in the duodenum and jejunum at postprandial concentrations and, thus the authors suggested that CCK may be involved in the hyperemia. This hypothesis is supported by the findings of Fara and Madden (37). They showed that intraarterial infusion of CCK into the autoperfused SMA vascular bed of anesthetized cats increased mucosal flow at the expense of submucosal flow. Because the increased intestinal flow after meals is confined to the mucosal layer of the intestinal wall (26, 43), CCK, but not secretin or gastrin, may contribute to the postprandial intestinal hyperemia.

There are many other naturally occurring substances which are vasodilators in the intestinal vascular bed. Serotonin (5-HT) dilates the small blood vessels of the intestine when locally infused intraarterially in anesthetized cats and dogs (6, 100). Serotonin is present in the gut wall, and its concentration in the portal blood increases after feeding or after intraduodenal instillation of 2778 mM glucose (11, 67, 73). Because serotonin is a vasodilator and has been shown to be released into the intestinal circulation by nutrients present in the gastrointestinal tract, it could be involved in the postprandial hyperemia. Indeed, Biber et al. (7) suggest that the vasodilator effect of CCK may be mediated through serotonin. In anesthetized cats, they abolished the vasodilator effects of i.a. administration of CCK by previous administration of dihydroergotamine (a 5-HT antagonist) or by making the intestinal vasculature tachyphylactic to 5-HT. Bradykinin concentration has also been shown to increase in the portal blood after instillation of 2778 mM glucose into the duodenum of conscious dogs (73). In anesthetized dogs, intraarterial infusion of

bradykinin dilates the intestinal vascular bed (52, 92). Kinins have been implicated as the mediators of functional vasodilation in the pancreas (57), thus it is quite possible that bradykinin could also be involved in the postprandial intestinal hyperemia.

Another mechanism by which intestinal blood flow could increase after a meal is via an increase in mucosal cell metabolism. Sit et al. (97) have shown than an increase in oxygen consumption of a jejunal segment accompanies the increase in flow when a mixture of the major constituents of chyme (digested food, bile, pancreatic enzymes) are placed in the lumen. Brodie et al. (17) have shown that there is an increase in oxygen uptake as well as blood flow to an isolated intestinal segment after instillation of a peptone solution. Also, Varro et al. (102) have shown that intrajejunal placement of isotonic glucose or glycine solutions increase local jejunal blood flow and oxygen consumption. These data suggest that there is an increase in intestinal aerobic metabolism as well as blood flow when chyme or nutrients are present intraluminally.

An increase in cellular aerobic metabolism could reduce local tissue concentration of oxygen and increase the levels of carbon dioxide, hydrogen ion, adenine compounds and other metabolites (53). These metabolites have been shown to be vasodilators and have been implicated in the functional hyperemia occurring in skeletal muscle during exercise (53, 54) and in reactive hyperemia in the heart (37). Bean and Sidky have studied the effects of lowering blood O_2 and raising blood CO_2 on local intestinal blood flow (5, 79). They showed that perfusion of an isolated intestinal loop with hypoxic blood (7% O_2) increases blood flow through the loop (5). In the same preparation, perfusion of the segment with hypercapnic blood (6.7%

CO_2) increases blood flow while perfusion with hypocapnic blood (0% CO_2) decreases local blood flow (79). Dabney et al. (29) have shown that local i.a. infusion of adenosine in a naturally perfused or constant flow preparation of an isolated ileal segment decreases vascular resistance. Mailman et al. (74), have shown that i.a. infusion of adenosine, AMP, c-AMP, and ATP into a side branch of the SMA increased blood flow through the SMA. From these data it would seem that changes in local mucosal concentration of various metabolites subsequent to an increase in local metabolism may be involved in the postprandial hyperemia. Because the concentrations of various metabolites in the blood draining the intestine after meals have not been firmly established, there is no way of knowing whether the responses to these vasodilator metabolites are physiological or not.

Nutrients in the lumen can increase intestinal blood flow by one or more of the above discussed mechanisms (neural, humoral, or metabolic). Neural and humoral mechanisms may involve the interaction of luminal nutrients with specific mucosal endocrine cell receptors or local nerve endings. An increase in mucosal cell metabolism may be a result of the energy-requiring active absorption of nutrients (e.g., glucose and amino acid absorption) and/or intracellular biotransformation of the absorbed nutrients (e.g., synthesis of triglycerides from absorbed monoglycerides and fatty acids). Another possibility is that the nutrients could produce a hyperemia through a direct vasodilator action on local blood vessels after their absorption. It is difficult to review all of the literature on the absorption of the end products of carbohydrate, protein and lipid digestion,

thus only aspects of the absorptive processes pertinent to the present study have been reviewed here.

Intestinal absorption of hydrolytic products of food digestion can be divided into two distinct processes: absorption of lipid soluble substances and absorption of water soluble substances. The major end products of luminal lipid digestion (monoglycerides and fatty acids) are lipid soluble. The major end products of carbohydrate digestion (glucose and saccharides) and protein digestion (amino acids and peptides) are water soluble.

The major end products of triglyceride digestion, monoglycerides and fatty acids, can pass through the lipid portion of the mucosal cell membrane by simple diffusion and, thus, there is no need to postulate the existence of a "carrier". The medium- and long-chain lipids (greater than 6 - 8 carbon atoms) are poorly soluble in water and their passive diffusion from the gut lumen into the mucosal cell is limited by the presence of an unstirred water layer overlying the epithelial mucosal cells (109, 110, 112). In the unstirred water layer, transport of molecules from the bulk intestinal contents to the mucosal cell occurs only by diffusion, and this layer is rate-limiting for passive diffusion of long chain fatty acids (94, 108, 109). The absorption of these long chain lipids is enhanced if bile acids are also present in the lumen (61, 62, 93, 108, 109). Bile acids can form micelles which greatly enhances the absorption of fatty acids and monoglycerides by solubilizing the lipids and allowing them to overcome the resistance to diffusion offered by the unstirred water layer (108, 109). The short chain lipids, on the other hand, are soluble in water and the unstirred water layer offers less resistance to their diffusion. Their absorption is not appreciably enhanced by bile acid micelles (31, 109).

Once inside the cell, most of the long-chain lipids, some of the medium-chain lipids, and few of the short-chain lipids are reesterified into triglycerides (16, 31, 66). The reesterification process is an energy consuming one, in that, ATP is required and the process does not occur in the presence of metabolic inhibitors (66). The newly synthesized triglycerides are enveloped in a lipoprotein coat and enter the lymph as chylomicrons (31, 34, 66). The fatty acids which are not reesterified pass into the portal circulation (31, 66).

Oligosaccharides are not absorbed to any appreciable extent by the mucosal cell (49). Instead, they are further hydrolyzed to monosaccharides by oligosaccharidases located in the brush border of the mucosal cell and the monosaccharides (mostly glucose) are then absorbed (19, 31, 49). Glucose is not lipid soluble and is too large to move through the cell membrane by simple diffusion. Thus, the existence of membrane bound carrier macromolecules which bind with glucose and, in some manner, translocate it to the interior of the cell has been postulated (19, 31, 49, 72). There is some evidence to suggest that these macromolecules may be the disaccharidases themselves (19). There is a structural requirement for glucose binding to this carrier in that the binding sites on the glucose molecule are thought to be C-1, C-2, C-4 and C-6. Substitutions at these sites diminish or abolish its active absorption (72).

The absorption of glucose appears to be dependent on extracellular sodium concentrations, and the sodium ion greatly facilitates glucose uptake into the mucosal cell (19, 27, 28, 31, 49, 72). The widely accepted theory on the mechanism of sugar transport, the "ion gradient theory", is based on this relationship between sodium and glucose absorption (27, 28,

72). Briefly, a carrier macromolecule in the brush border has two sites, one for sodium ion and the other for glucose. The glucose and sodium ions are translocated to the inside of the cell where the sodium concentration is low. An active transport system (involving Na^+-K^+ ATPase and probably located in the basolateral membrane) then moves Na^+ out of the cell, thus maintaining the low intracellular sodium concentration. Thus, active transport of glucose is accomplished by the existent gradient for sodium ion (greater outside than inside of the cell) which is in turn maintained by the energy requiring Na^+-K^+ ATPase system (24, 49, 72). In addition to being Na^+-dependent, the entrance of glucose into the mucosal cell is inhibited by phlorizin and exhibits saturation kinetics (19). Once inside the mucosal cell about 10% of the glucose is metabolized (31) and the remainder probably leaves the basal portion of the cell by specific transport process which is energy- and Na^+-independent and shows less inhibition which phlorizin than the brush border transport process (9, 35, 68).

In contrast to oligosaccharides, some oligopeptides are absorbed intact by the mucosal cell and then hydrolyzed to their constituent amino acids by intracellular oligopeptidases (48, 72, 76, 77). The absorption of some di- and tripeptides, but not tetrapeptides has been demonstrated and the process of peptide absorption appears to be active, Na-dependent, and exhibits competition among peptides for absorption (76, 77). Other oligopeptides are hydrolyzed by oligopeptidases of the brush border to their constituent amino acids and then absorbed into the mucosal cell (48, 83). Amino acids, like glucose, are actively absorbed via an interaction with a specific transport macromolecule which translocates the amino acids across the brush border into the mucosal cell (48, 72, 113). Four different

transport systems have been postulated: neutral, basic, acidic, and imino (48, 72, 113). The neutral transport system has been studied extensively and there appears to be a structural requirement for interaction of amino acids with the carrier (48, 72, 99, 113). The carboxyl group on the alpha carbon is mandatory for transport, and an unsubstituted NH_2 and non-polar side chain on the alpha carbon are preferred. Extracellular Na^+ is required for amino acid transport (as is the case for glucose absorption), and thus, the ion gradient theory has been postulated as the mechanism for active absorption of amino acids (27, 48, 72, 99, 113). Once inside the mucosal cell only two amino acids are metabolized to any extent; a fraction of glutamic and aspartic acids undergo transamination with pyruvate to form alanine, CO_2 and other metabolites (78, 99, 111, 113). Absorbed amino acids leave the mucosal cell by a transport mechanism similar to that postulated for glucose (35).

An interesting feature of the transport processes involved in absorption of glucose and amino acids is the apparent interaction between amino acid and sugar transport (3, 10, 90). It appears that the absorption of amino acids by the mucosal cell is inhibited by sugars and vice versa (3, 10, 90). The mechanisms of this interaction is unknown, but several hypotheses have been put forth (90) and of these the "allosteric-interaction hypothesis" has the greatest support. This hypothesis assumes that the various carriers are not entirely independent or unrelated, and that binding of glucose to its carrier in some manner influences the binding of amino acid to its own carrier.

Even in the case of the water soluble nutrients the unstirrred water layer has a profound effect on their absorption. As discussed earlier,

molecules can move through this layer only by diffusion. The resistance of this layer causes a concentration gradient to develop between the bulk luminal contents and the mucosal cell. The untirred water layer can reduce the apparent permeability coefficient of a passively absorbed substance and increase the apparent Michaelis constant of an actively transported substance (33, 49, 72, 110). Stirring or shaking the medium of everted intestinal sacs decreases the Km for active transport and increases the permeability coefficient for passively absorbed substances by decreasing the thickness of the unstirred water layer (110, 112). Although bile or bile salts have been shown to play an important role in absorption of lipids by decreasing the resistance to diffusion offered by the unstirred water layer (108, 109), the information available on the effect of bile or bile salts on glucose and amino acid transport is controversial. In vitro, conjugated bile salts do not alter glucose or amino acid transport by the intestinal mucosa (115). In vivo, conjugated bile salts (5 - 10 mM) either had no effect (46) or inhibited (55, 91) the absorption of glucose (10 - 20 mM by the rat jejunum). Whole hepatic bile did not alter absorption of 10 mM glucose, but delipidated bile inhibited glucose absorption from rat jejunal loops in vivo (91). The concentration of glucose used in these studies is less than that found to be present in the gut after meals (14).

CHAPTER III

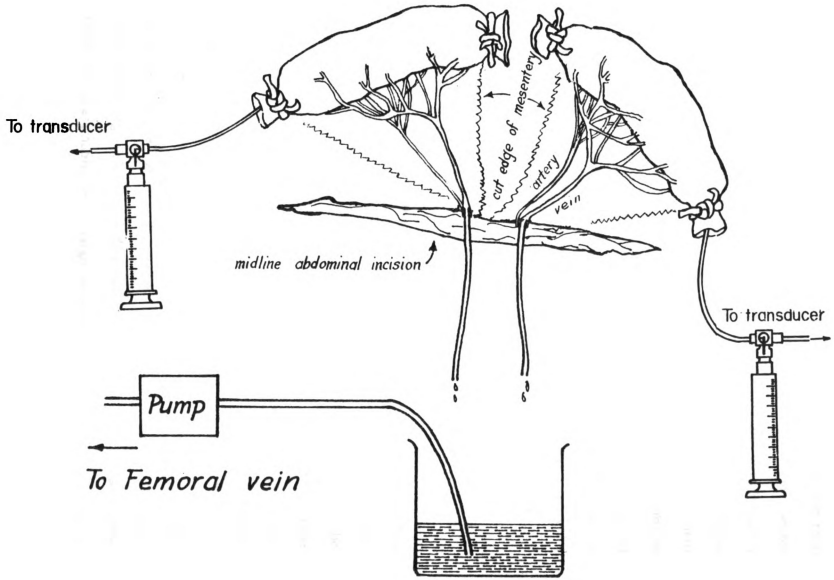
METHODS AND MATERIALS

Surgical Preparation

All experiments were conducted on mongrel dogs (13 - 29 kg) of either sex which were fasted for 24 hours. The dogs were anesthetized by an intravenous administration of chloralose (75 mg/kg) and urethan (500 mg/kg). They were ventilated with a positive-pressure respirator (Harvard, Model 607, Dover, Mass.) at a rate and volume which maintained a normal arterial blood pH (pH, 7.38 7.43). Heparin sodium (6 mg/kg) was given intravenously as an anticoagulant. Systemic arterial pressure was continuously monitored with a pressure transducer (Statham, p23Gb, Hato Rey, Puerto Rico) attached to a polyethylene cannula (i.d. = 2.2 mm; o.d. = 3.3 mm) in a femoral artery.

The abdominal cavity was exposed via a midline incision and a loop of jejunum about 30 cm from the ligament of Treitz was exteriorized. As shown in Figure 1, the loop was divided into two segments of equal length such that each segment was drained by a single vein. The veins were cannulated with polyethylene tubing and the venous outflows were directed into a reservoir which initially contained 6 percent dextran in normal saline. The venous blood was continuously returned to the animal by a Holter pump (Extracorporeal Medical Specialties, Inc., Model RE 161, King

1. Double segment preparation of the canine jejunum.


To transducer
cut edge of mesentery
artery
vein
midline abdominal incision
To transducer
Pump
To Femoral vein

of Prussia, Pa.) via a femoral vein at a rate equal to that of the total venous outflow. A rubber tube (i.d. = 3.0 mm; o.d. = 5.0 mm) was inserted into the lumen of each segment for the introduction and withdrawal of solutions. At all other times the tubes were connected to pressure transducers (Statham, p23Gb, Hato Rey, Puerto Rico) for the measurement of intraluminal pressures. Both ends of each segment were tied and the mesentery cut to exclude collateral flow. The two segments were covered with a plastic sheet to minimize tissue dehydration and kept at 37°C with a heat lamp. Both systemic arterial and intestinal lumen pressures were recorded on an oscillograph (Sanborn, Model 7714-09A, Waltham, Mass.).

Test Solutions

The composition of the nutrient solutions studied is listed in Table 1. Glucose, dipeptides, amino acids, lipids, and taurocholic acid were studied at physiological concentrations; i.e., approximating those found in the lumen of the jejunum after meals (2, 14, 40, 59, 82, 83, 85). The amino acids were also studied at higher concentrations (Table 1, I, D 2) and 3)). All of the amino acid mixtures contained amino acids in the proportion found in beef muscle (98).

The concentration of a bile salt, taurocholic acid, used in the lipid solutions is above the critical micellar concentration (62, 69). The solutions containing oleic acid and/or monoolein with taurocholic acid were clear and presumably had a large micellar phase; whereas, solutions of these lipids without taurocholic acid were opaque and presumably had no micellar phase. The solutions of caproic acid (a short chain fatty acid)

Table 1. The composition of the test solutions

I. Basic Nutrients.

A. A mixture of the following nutrients:

1) Glucose (150),

2) 16 amino acids (25.2): ile (2.1), leu (3.2), met (1.0), phe (1.3), thr (2.0), trp (0.3), val (2.4), lys (2.0), arg (1.1), asp (2.0), cystine (0.2), glu (2.8), gly (2.1), his (0.5), pro (1.7), and tyr (0.5),

3) Micellar lipids (70): oleic acid (40), monoolein (20), and taurocholic acid (10).

B. Glucose (150).

C. Amino Acids.

1) A mixture of 16 amino acids (25.2): concentration of each amino acid same as in A.

2) Solutions of amino acids at concentrations 10 times those in A.

a. A mixture of 16 amino acids (252).
b. A mixture of the 8 essential amino acids (143): ile, leu, met, phe, thr, trp, val, and lys.
c. A mixture of the 8 nonessential amino acids (109): arg, asp, cystine, glu, gly, his, pro, and tyr.
d. A mixture of glu and asp (48).
e. A mixture of the 6 nonessential amino acids (61): arg, cystine, gly, his, pro, and tyr.
f. The 16 amino acids individually.

3) Solutions of amino acids at concentrations 5 times those in A.

a. Glu at 5 x the concentration as in A.
b. Asp at 5x the concentration as in A.

D. Micellar and non-micellar lipids.

1) Oleic acid (40), monoolein (20), and taurocholic acid (10).

2) Oleic acid (40) with and without taurocholic acid (10).

3) Monoolein (20) with and without taurocholic acid (10).

4) Caproic acid (20) with and without taurocholic acid (10).

5) Taurocholic acid (10).

continued

Table 1. Continued

II. Basic nutrients with and without bile.
A. Glucose (200) with and without 10 and 33% bile.
B. A mixture of 16 amino acids (25.2) with and without 10 and 33% bile.
C. A mixture of the dipeptides, gly-asp (14.2) gly-glu (14.4) and pro-leu (8.9) with and without 10% bile.
D. Oleic acid (40) with 33% bile.
E. Oleic acid (20) with and without 10 and 33% bile.
F. Caproic acid (20) with and without 10 and 33% bile.
G. Triolein (10) with and without 10% bile.
H. Triolein (10) and pancreatic enzymes with and without 10% bile.
I. 10 and 33% bile.

Number in parenthesis denotes concentrations in mM/lit.

were clear whether or not taurocholic acid was present. To facilitate mixing, all of the lipid solutions were sonified with a sonic dismembrator (Model 300, Artek System Corp., Farmingdale, N.Y.).

Two different concentrations of bile were used in the study. The bile was aspirated from the dog's gall bladder just prior to each experiment. The solutions containing bile alone were made by diluting 1 volume of gall bladder bile with 2 volumes of normal saline (33% bile) or 9 volumes of normal saline (10% bile). The glucose, amino acids, or lipid solutions containing bile were prepared such that the concentration of bile in these solutions was the same as that in the solution containing bile alone. Also, the concentrations of these nutrients in the solutions containing bile were the same as those in the nutrient solution containing no bile.

Some of the test solutions listed in Table 1 were isotonic when prepared, others were hypotonic. An appropriate amount of sodium chloride was added to the hypotonic solutions to make them nearly isotonic (range, 267 to 350 mOsm/kg). The pH of all solutions was adjusted with either 3 M NaOH or 3 M HCL to a value between 6.5 and 7.4. The test solutions were prepared on the day of the experiment and kept in a water bath at 37^{o}C.

Oleic and caproic acids (United States Biochemical Corporation, Cleveland, Ohio), triolein, monoolein and taurocholic acid, (Sigma Chemical Company, St. Louis, Missouri) were all greater than or equal to 95% pure. Glucose (Mallinckrodt Chemical Works, St. Louis, Missouri), the amino acids (Sigma) and the dipeptides (United States Biochemical Corporation) were in the purest form available from the respective suppliers. Carbon-14

labelled oleic acid (oleic acid-1-^{14}C) was obtained from New England Nuclear and was certified as 98% pure.

Experimental Protocol

Using the double jejunal segment preparation (Figure 1), three series of experiments were performed. The protocol for the experiments consisted of three successive 15 minute periods; precontrol, test, and postcontrol. In the pre- and postcontrol periods both segments contained 10 ml of normal saline. The luminal contents in the test period varied depending on the purpose of the experiment. In some experiments, one segment contained 10 ml of a test solution while the other segment contained 10 ml of normal saline. Thus, the vascular effects of the test solution in one segment could be studied using saline as a control in the other segment. When the vasoactivities of two test solutions were being compared, one segment contained one of the two solutions and the other segment contained the other test solution. During each fifteen minute period venous outflows from both segments were simultaneously collected in graduated cylinders in 3-min samples with 1-min intervals between collections. After each period, the lumen contents were withdrawn and the lumen was gently and thoroughly rinsed with normal saline. No more than four or five test solutions were studied in a given animal.

Series 1

The first aim of this series of experiments was to systematically determine which of the major hydrolytic products of protein, carbohydrate, and lipid digestion increase local jejunal blood flow. The vascular effects of the following test solutions were studied using a protocol in which one segment contained normal saline while the other segment contained a test solution during the test period:

1) A mixture containing glucose, 16 amino acids (25.2 mM), and micellar lipids,
2) Glucose,
3) A solution containing 16 amino acids (25.2 mM),
4) Micellar lipids (taurocholate, monoolein and oleic acid), and
5) Taurocholate.

Since the solution containing 16 amino acids at 25.2 mM/liter did not affect blood flow when placed in the jejunum, further studies were carried out to see if amino acids at higher concentrations would increase flow. Intraduodenal perfusion of these 16 amino acids at 182 mM/liter has been shown to release CCK, a vasodilator hormone, from the gut wall (45). Intrajejunal placement of 252 mM mixture of these 16 amino acids did, indeed, increase flow (Table 3). Therefore, subsequent experiments were performed to determine which of the 16 amino acids were vasoactive at these concentrations. Initially, the vasoactivities of 8 essential and 8 nonessential amino acids were compared. The latter solution increased flow but the former solution did not. Thus, the 8 nonessential amino acids were further divided into a solution containing glutamic (glu) and aspartic

(asp) acids and a solution containing the remaining 6 nonessential amino acids and their vasoactivies compared. The former solution increased flow but the latter solution did not. Finally, the vascular effects of each of the 16 amino acids were studied individually.

Bile salts play an important role in lipid digestion and absorption. Thus, the next aim of this series of experiments was to see if taurocholic acid, a bile salt, would influence the vasoactivity of lipids. Thus, the vasoactivities of the following three pairs of solutions were compared by placing one of the paired solutions in one segment and the other solution in the other segment during the test period.

1) 40 mM oleic acid with 10 mM taurocholic acid and 40 mM oleic acid alone,
2) 20 mM monoolein with 10 mM taurocholic acid and 20 mM monoolein alone, and
3) 20 mM caproic acid with 10 mM taurocholic acid and 20 mM caproic acid alone.

Series 2

The aims of this series were to see 1) if dipeptides were vasoactive, 2) if addition of 10 or 33% bile to fatty acids, amino acids, dipeptides, or glucose would alter the vascular effects of these nutrients, 3) if, in the presence of bile, the vasoactivities of oleic acid (a long chain fatty acid) and caproic acid (a short chain fatty acid) differ and 4) if a more concentrated fatty acid solution produces a greater vasodilation. The vasoactivities of the following paired solutions were compared.

1) 10 and 33% bile and normal saline,

2) 20 mM oleic acid with 10 and 33% bile and 20 mM oleic acid alone,

3) 20 mM caproic acid with 10 and 33% bile and 20 mM caproic acid alone,

4) A mixture of 16 amino acids at 25.2 mM with 10 and 33% bile and the 16 amino acids alone,

5) A mixture of three dipeptides at 37.5 mM with 10% bile and the three dipeptides alone,

6) 200 mM glucose with 10 and 33% bile and 200 mM glucose alone,

7) 20 mM oleic acid with 33% bile and 20 mM caproic acid with 33% bile, and

8) 20 mM oleic acid with 33% bile and 40 mM oleic acid with 33% bile.

Because only oleic acid, a long chain fatty acid, increased flow in the presence of both 10 and 33% bile, additional experiments were performed to determine whether luminal triglycerides, containing oleic acids, must be digested before the hyperemic response to the lipids occurs. The vasoactivities of the following paired solutions were compared:

1) Triolein with 10% bile and triolein alone, and

2) Digested triolein with 10% bile and digested triolein alone.

The two digested triolein solutions were prepared by adding 100 mg of a pancreatic enzyme preparation (Viokase, Viobin Corp., Monticello, Ill.) to 25 ml of a solution containing triolein and bile and 25 ml of a solution containing triolein alone. To permit digestion, the two solutions were

stirred with magnetic stirrers for at least 30 minutes before they were placed in the jejunal lumen.

Series 3

The results of the second series of experiments indicated that the vasoactivites of luminal glucose and oleic acid were affected by the presence of bile. The aim of the third series of experiments was to determine if bile influences the vascular effects of these two nutrients by altering their absorption rate. The protocol for these experiments was similar to the one used for the first two series of experiments. For the glucose experiments the protocol consisted of three periods (precontrol, test, and postcontrol) and for the fatty acid experiments the protocol consisted of two periods (precontrol and test). In all experiments of this series, the absorption rate of the nutrients were measured along with blood flow.

Glucose Absorption. The effect of 10 and 33% bile on glucose absorption was assessed by placing 200 mM glucose in one segment and 200 mM glucose with bile in the other segment and comparing the glucose absorption from both segments. Glucose absorption is an active process involving the expenditure of energy (49, 72). Thus in some experiments (33% bile) the oxygen consumption of the two segments were also measured and compared.

The glucose absorption from each segment was defined as the product of jejunal blood flow and the V-A glucose difference. Blood samples for

the determination of glucose content were simultaneously taken from a femoral artery and both veins draining the two jejunal segments. During the control periods these samples were taken 15 minutes after placement of normal saline. During the test period these were taken at 7, 11, and 15 minutes after the placement of the glucose solutions. The blood samples were centrifuged and a known volume of plasma removed for analysis of glucose content. Glucose content was determined within 24 hours by two methods. In the experiments in which 33% bile was used plasma glucose content was determined colorimetrically with the Glucostat Reagent Set (Worthington Biochemical Corporation, Freehold, New Jersey). Blood glucose concentration was then determined from a nomograph using the measured values of plasma glucose concentration and hematocrit. In the experiments in which 10% bile was used, blood glucose content was determined with a YSI Model 23A Glucose Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio).

The oxygen consumption by the two jejunal segments was calculated as the product of jejunal blood flow and the A-V_{O_2} difference. The oxygen content of the arterial (femoral) blood was measured at the end of each of the three periods (precontrol, test, and postcontrol). Blood samples for the determination of venous blood oxygen content were taken once during the control periods and twice during the test period. During the control periods these samples were taken randomly, from one segment at 11 minutes and from the other segment at 15 minutes after placement of normal saline. During the test period these samples were taken randomly from one segment at 3 and 11 minutes and from the other segment at 7 and 15 minutes after the placement of the glucose solutions. The blood oxygen

contents were determined by a Lex O_2 Con-TL (Lexington Instruments, Waltham, Massachusetts).

Oleic Acid Absorption. The effect of 10% bile on oleic acid absorption was assessed by placing oleic acid alone in one segment and oleic acid with bile in the other segment for 15 minutes and then comparing the amount of fatty acid absorbed by the two jejunal segments. Radioactively labelled oleic acid was used as a tracer and was added to each solution before placement. The difference in the amount of radioactivity in the placed and recovered solutions, as well as, the amount of radioactivity in the mucosal layer were used as indices of fatty acid absorption from the lumen of each segment.

To both 20 mM oleic acid solutions (with and without bile) tracer amounts (10 - 20μl) of oleic acid-1-^{14}C were added and the solutions were resonified to insure adequate dispersion of the label. After sonification, six samples (200μl) were obtained from each solution and placed in scintillation vials for subsequent determination of radioactivity. Within 5 minutes after sonification, 10 cc of the oleic acid solution was placed in one segment and 10 cc of the oleic acid-bile solution was placed in the other segment. Fifteen minutes later the solutions were removed, the volume recorded, and 10 samples (200μl) were obtained from each recovered solution and placed in scintillation vials for subsequent measurement of radioactivity.

Immediately after the fatty acid solutions were removed from the lumen of both segments, the two segments were excised, weighed, rinsed in normal saline, and blotted on filter paper. The mucosal layer was

separated from the submucosal and muscle layers with a blunt instrument. Mucosal samples (100 - 250 mg) were placed into tared scintillation vials, and the vials were reweighed to determine wet tissue weight. The mucosal samples were digested by adding 100μl of 60% perchloric acid to the vials and incubating them at 60 - 70^{o} for 4 hours in a Dubnoff Metabolic Shaking Incubator (Precision Scientific, Chicago, Ill.). After digestion, 200μl of 30% hydrogen peroxide was added to decolorize the samples. At this time the samples of the placed and recovered oleic acid solutions were also decolorized. All samples were then allowed to stand in the dark for 8 hours at room temperature. Ten ml of a liquid scintillation cocktail (Ready-Solv HP, Beckman) was added to each vial. The samples were counted in a liquid scintillation counter with automatic quench correction (Beckman Instruments). The counts obtained were corrected for quenching using the external external standard channels ratio method. The amount of oleic acid lost from the lumen was calculated as follows: (dpm/200μl sample of placed solution x total volume of placed solution) - (dpm/200 μl sample of recovered solution x total volume of recovered solution). The amount of dpm accumulated in the mucosa was calculated as follows: dpm/gm mucosal sample x segment weight x 0.631. The term, 0.631, represents the percent of total jejunal wall weight attributed to mucosa, i.e., 63.1% (114).

Bile contains bile salts which solubilize long chain lipids by forming micelles (60, 61, 62). Long chain lipids can also be effectively solubilized in an aqueous solution without bile by raising the pH of the solution (60). Thus, additional experiments were performed to determine if the effects of bile on the vasoactivity and absorption of oleic acid could be mimicked by simply raising the pH of an oleic acid solution. The protocol was identical

to that described above except that the pH's of both the oleic acid and oleic acid-bile solutions were adjusted to 9.3 - 9.6. As a control, normal saline at pH 9.5 was placed in the lumen prior to placement of the two oleic acid solutions.

Treatment of Data

After each experiment the jejunal segments were weighed and all blood flow and vascular resistance data were normalized to 100 gm tissue. Glucose absorption and oxygen consumption rates were also normalized to 100 gm tissue. As there was no apparent pattern among the values for A-V glucose, A-V oxygen, glucose absorption, or oxygen consumption obtained during the test periods, the average of these values were used to present the data. Fatty acid absorption data were expressed as a percent of placed fatty acid. All of the data were analyzed using the Student's t-test for paired or unpaired comparison.

CHAPTER IV

RESULTS

Systemic arterial pressure (range, 95 to 167 mm Hg) was not significantly altered by intrajejunal placement of either normal saline or any nutrient solution. Lumen pressure which ranged from 0 to 11 mm Hg after placement of normal saline was not signficantly altered by the placement of any nutrient solution.

When the venous outflow was increased after luminal placement of a nutrient solution, the increase in flow followed the general pattern illustrated in Figure 2. As shown, after placement of a mixture of glucose, amino acids and micellar lipids, the venous outflow gradually increased, reached a plateau within 8 - 11 minutes and remained at that level for the next four minutes. Because all of the nutrients which increased flow exhibited a similar pattern, the venous outflows measured at 12 - 15 minutes after placement of nutrients or normal saline were used to present the data unless specifically stated otherwise.

Series 1

The vascular effects on intrajejunal placement of 150 mM glucose, a 25.2 mM mixture of 16 amino acids (ile, leu, met, phe, thr, trp, val, lys, arg, asp, cystine, glu, gly, his, pro, and tyr), and micellar lipids (40 mM oleic acid, 20 mM monoolein, and 10 mM taurocholate) are shown in Table

2. Percent change from precontrol values of blood flow through two jejunal segments after luminal placement (arrow) of a mixture of glucose, 16 amino acids and micellar lipids (G-AA-ML) in one segment (- -o- -) and normal saline in the other segment (—o—).

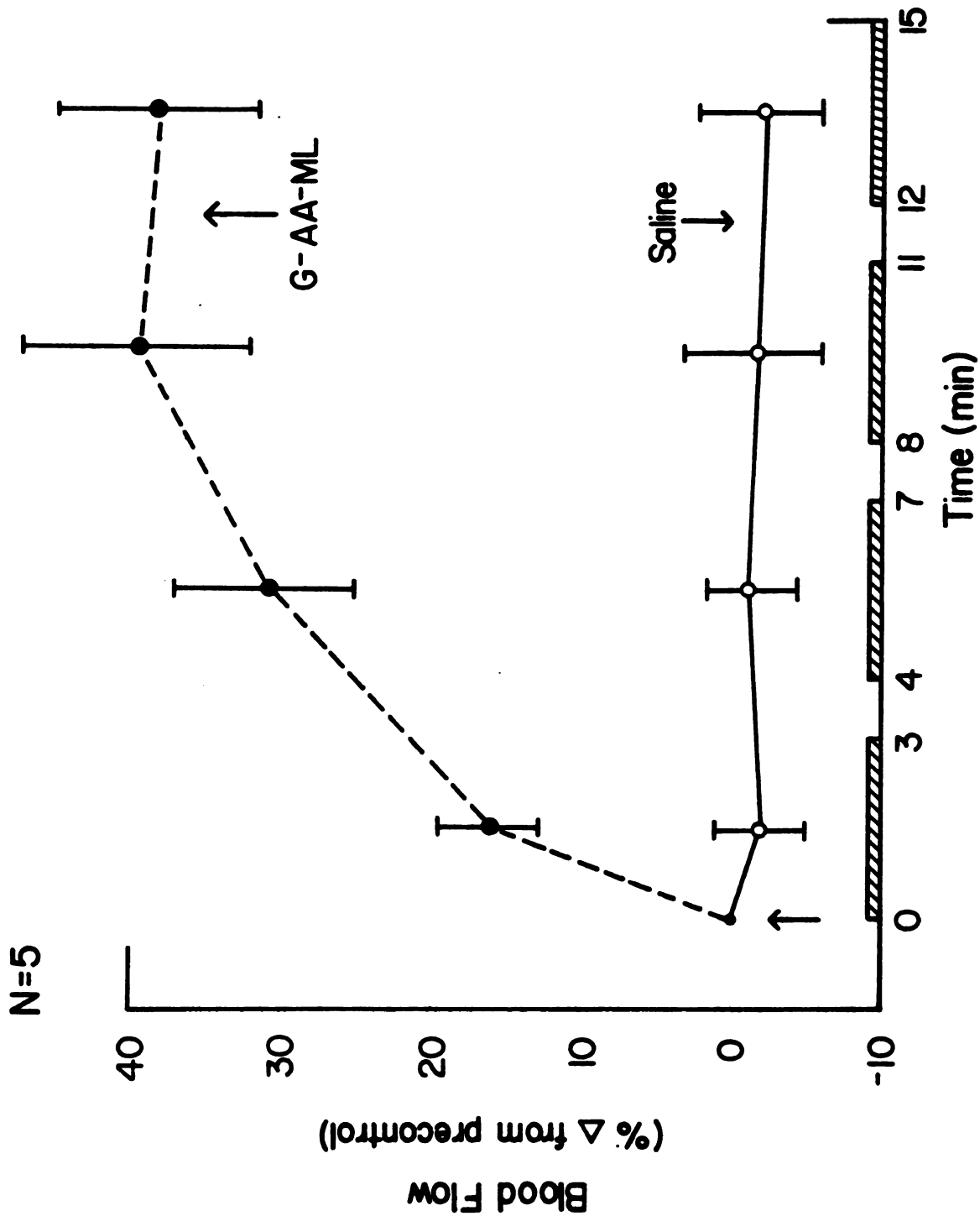

Blood Flow
(% Δ from precontrol)
40
30
20
10
0
-10
N=5
0
3
4
7
8
11
12
15
Time (min)
G-AA-ML
Saline

Table 2. Effects of luminal placement of 150 mM glucose, 25.2 mM mixture of 16 amino acids (16 AA)+ and 60 mM micellar lipids+ on local jejunal blood flow and vascular resistance.

	Segment A			Segment B			(N)
	Precontrol	Test	Postcontrol	Precontrol	Test	Postcontrol	
	Saline	Glucose + 16 AA + Micellar Lipids	Saline	Saline	Saline	Saline	(5)
Blood Flow	47.9 ± 9.7	65.8 ±14.2*	50.1 ± 7.5	49.9 ± 8.9	47.8 ± 7.5	46.3 ± 6.8	
Resistance	3.3 ± 0.6	2.4 ± 0.3*	2.8 ± 0.3	3.1 ± 0.5	3.0 ± 0.3	3.0 ± 0.3	
	Saline	Glucose	Saline	Saline	Saline	Saline	(6)
Blood Flow	72.9 ± 8.0	77.5 ± 8.2*	71.0 ± 7.6	69.6 ±10.9	68.7 ± 8.6	69.2 ± 9.4	
Resistance	1.8 ± 0.2	1.7 ± 0.2*	2.0 ± 0.3	2.0 ± 0.3	2.1 ± 0.3	2.1 ± 0.4	
	Saline	16 AA	Saline	Saline	Saline	Saline	(7)
Blood Flow	48.1 ± 5.5	47.3 ± 5.1	46.9 ± 4.5	47.2 ± 5.7	46.7 ± 5.4	47.7 ± 4.2	
Resistance	3.3 ± 0.3	3.3 ± 0.3	3.3 ± 0.3	3.4 ± 0.4	3.4 ± 0.4	3.2 ± 0.3	
	Saline	Micellar Lipids	Saline	Saline	Saline	Saline	(7)
Blood Flow	61.5 ± 7.9	75.9 ± 8.0*	59.9 ± 8.3	59.0 ± 5.7	57.6 ± 5.8	57.7 ± 6.4	
Resistance	2.4 ± 0.3	1.9 ± 0.2*	2.4 ± 0.3	2.4 ± 0.2	2.5 ± 0.2	2.5 ± 0.3	

Values are means ± S.E. Blood flow = ml/min/100 gm. Resistance = mm Hg/ml/min/100 gm.

+ 16AA: see Table 1 for constituent amino acids and their concentrations.
Micellar lipids: 40 mM oleic acid, 20 mM monoolein, and 10 mM taurocholate.

* Value is significantly different from the precontrol value of each segment ($p < 0.05$, paired Student's t-test).

2. Placement of a mixture of glucose, 16 amino acids and micellar lipids significantly increased local jejunal blood flow (+38.2 ± 6.8%) and decreased vascular resistance (-26.8 ± 4.5%). When glucose, a mixture of 16 amino acids, and micellar lipids were placed in the lumen individually, only glucose and the micellar lipids increased flow (+6.4 ± 1.0% and +26.3 ± 5.1%, respectively) and decreased vascular resistance (-6.0 ± 1.1% and −19.7 ± 2.9% respectively). Placement of a mixture of the 16 amino acids did not significantly alter flow or resistance. There were no significant changes in flows or resistances in the respective control segments (Table 2, Segment B) which contained normal saline during the control and test periods.

Although the 25.2 mM mixture of 16 amino acids did not increase flow (Table 2), a 252 mM mixture of the 16 amino acids did increase flow and decrease resistance (Tables 3 and 4). Further studies were, thus, undertaken to determine which of the amino acids were vasoactive at these higher concentrations. The 16 amino acid mixture contained 8 essential amino acids (isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine, and lysine) and 8 nonessential amino acids (arginine, aspartic acid, cystine, glutamic acid, histidine, proline, and tyrosine). As shown in Tables 3 and 4, the mixture of nonessential amino acids increased flow and decreased resistance, while the mixture of essential amino acids did not significantly alter flow or resistance.

The mixture of 8 nonessential amino acids contained the dicarboxylic amino acids, glutamic and aspartic acids. Glutamic and aspartic acids are the only two amino acids which are extensively transaminated in the mucosa during their absorption (48, 78, 99, 113). Due to this peculiar

Table 3. Effects of luminal placement of amino acids[+] at various concentrations on local jejunal blood flow and vascular resistance.

	Segment A			Segment B			(N)
	Precontrol	Test	Postcontrol	Precontrol	Test	Postcontrol	
	Saline	16 AA (252)	Saline	Saline	Saline	Saline	(8)
Blood Flow	53.3 ± 9.1	57.6 ± 9.0*	52.1 ± 8.7	57.2 ± 8.8	56.5 ± 8.6	54.3 ± 7.5	
Resistance	2.9 ± 0.5	2.6 ± 0.4*	2.9 ± 0.4	2.6 ± 0.3	2.6 ± 0.3	2.6 ± 0.3	
	Saline	8 NEAA (109)	Saline	Saline	8 EAA (143)	Saline	(6)
Blood Flow	46.2 ± 7.4	50.1 ± 7.4*	44.1 ± 7.2	50.3 ± 9.7	50.6 ± 9.3	47.8 ±10.0	
Resistance	2.9 ± 0.4	2.7 ± 0.4*	3.1 ± 0.4	2.8 ± 0.4	2.8 ± 0.4	3.0 ± 0.5	
	Saline	Glu + Asp (48)	Saline	Saline	6 NEAA (61)	Saline	(6)
Blood Flow	49.2 ± 4.0	53.9 ± 4.3*	47.2 ± 3.7	47.2 ± 3.5	47.5 ± 4.1	47.5 ± 3.3	
Resistance	3.0 ± 0.3	2.7 ± 0.2*	3.1 ± 0.3	3.1 ± 0.3	3.1 ± 0.3	3.1 ± 0.3	
	Saline	Glu (28)	Saline	Saline	Asp (20)	Saline	(6)
Blood Flow	49.0 ± 5.7	51.8 ± 6.3*	49.4 ± 5.4	45.0 ± 4.6	47.1 ± 4.5*	45.9 ± 4.2	
Resistance	3.2 ± 0.3	3.1 ± 0.3*	3.2 ± 0.3	3.5 ± 0.3	3.3 ± 0.3*	3.4 ± 0.3	
	Saline	Glu (14)	Saline	Saline	Asp (10)	Saline	(4)
Blood Flow	46.1 ± 3.0	45.3 ± 3.3	41.7 ± 2.8	44.2 ± 3.2	43.9 ± 4.0	41.6 ± 3.3	
Resistance	3.0 ± 0.3	3.1 ± 0.3	3.3 ± 0.4	3.1 ± 0.3	3.2 ± 0.3	3.3 ± 0.3	

Values are means ± S.E. Blood Flow = ml/min/100 gm. Resistance = mm Hg/ml/min/100 gm.

+ Amino acid concentrations, in mM per liter, are listed in parenthesis. 8 EAA = 8 essential amino acids, 8 NEAA = 8 nonessential amino acids, 6 NEAA = 6 nonessential amino acids, and 16 AA = 8 EAA + 8 NEAA. The individual amino acids and their concentrations in these mixtures are listed in Table 1.

* Value is significantly different from precontrol value of each segment ($p < 0.05$, paired Student's t-test).

Table 4. Percent changes from precontrol values of blood flow and vascular resistance of two jejunal segments after luminal placement of amino acids[+] at various concentrations.

	Segment A	Segment B	D = A - B[++]	(N)
	16 AA (252)	Saline		(8)
Blood Flow	+10.6 ± 3.1*	-0.5 ± 1.7	11.1 ± 2.9*	
Resistance	- 9.5 ± 2.9*	+0.7 ± 1.7	10.3 ± 2.1*	
	8 NEAA (109)	8 EAA (143)		(6)
Blood Flow	+ 9.7 ± 1.7*	+1.5 ± 1.1	8.2 ± 1.1*	
Resistance	- 8.6 ± 1.5*	-1.4 ± 1.0	7.2 ± 0.9*	
	Glu + Asp (48)	6 NEAA (61)		(6)
Blood Flow	+ 9.7 ± 1.5*	+0.2 ± 1.8	9.6 ± 2.1*	
Resistance	- 9.5 ± 1.3*	-1.5 ± 1.9	8.0 ± 2.4*	
	Glu (28)	Asp (20)		(6)
Blood Flow	+ 5.4 ± 0.6*	+4.7 ± 0.8*	0.7 ± 0.9	
Resistance	- 5.5 ± 0.7*	-5.1 ± 0.9*	0.4 ± 0.8	
	Glu (14)	Asp (10)		(4)
Blood Flow	- 1.8 ± 1.5	-0.9 ± 2.4	0.9 ± 2.4	
Resistance	+ 1.7 ± 2.2	+1.0 ± 3.6	0.8 ± 2.4	

Values are means ± S.E.

+ Amino acid concentrations, in mM per liter, are listed in parenthesis. 8 EAA = 8 essential amino acids, 8 NEAA = 8 nonessential amino acids, 6 NEAA = 6 nonessential amino acids, and 16 AA = 8 EAA + 8 NEAA. The individual amino acids and their concentrations in these mixtures are listed in Table 1.

++ D = A - B represents the difference in changes occurring in the two segments.

* Values are significant at $p < 0.05$ (paired Student's t-test).

property, glutamic and aspartic acids were omitted from the mixture of 8 nonessential amino acids and the vascular effects of a mixture of these two nonessential amino acids and the remaining 6 nonessential amino acids (arg, cystine, gly, his, pro, and tyr) were compared. As shown in Tables 3 and 4, the mixture of glutamic and aspartic acids increased flow and decreased resistance while the other 6 nonessential amino acids did not significantly alter flow or resistance.

To determine whether glutamic and aspartic acid are vasoactive when they are placed individually in the lumen, and if so, whether their effects are similar, the vascular effects of glutamic acid were compared to those of aspartic acid. As shown in Tables 3 and 4, both 20 mM aspartic acid and 28 mM glutamic acid increased blood flow and decreased vascular resistance. Glutamic acid increased flow by 5.4% and decreased resistance by 5.5%, while aspartic acid increased flow by 4.7% and decreased resistance by 5.1%. The difference in their vascular effects (0.7 ± 0.9 for blood flow and 0.4 ± 0.8 for resistance) was not significant (Table 4, D = A - B) indicating that the hyperemic effects of aspartic acid and glutamic acid are similar at the concentrations used here. However, both amino acids did not significantly alter blood flow or vascular resistance when their concentrations were reduced to half of those at which they produced vasodilation (Tables 3 and 4).

To insure that the lack of vascular effect of the mixture of 8 essential amino acids or the mixture of 6 nonessential amino acids was not due to some interaction among the amino acids, the 8 esssential and 6 nonessential amino acids were also tested individually. When these 14 amino acids were placed individually in the lumen at the same

concentrations as in the mixtures, each was found to be without significant effect on local jejunal blood flow or vascular resistance (N = 3 for each amino acid).

The micellar lipid solution which increased local jejunal blood flow when placed in the lumen contained taurocholate (a bile salt), monoolein (a long chain monoglyceride), and oleic acid (a long chain fatty acid). Therefore, experiments were designed to determine which of these three constituents of the micellar lipid solution was responsible for the hyperemia and the results are shown in Tables 5 and 6. Placement of taurocholate (10 mM) did not significantly alter local blood flow or vascular resistance. A mixture of taurocholate (10 mM) and monoolein (20 mM) increased flow and decreased resistance but monoolein alone did not have any vascular effects. Similarly, a mixture of taurocholate (10 mM) and oleic acid (40 mM) increased flow and decreased resistance while oleic acid alone did not alter flow or resistance. Tables 5 and 6 also show the vascular effect of a short chain fatty acid, caproic acid with and without taurocholate. Caproic acid (20 mM) did not significantly alter local jejunal blood flow or vascular resistance whether it was placed in the lumen alone or mixed with taurocolate (10 mM).

Series 2

A previous study has shown that bile markedly enhanced the vasodilator effect of digested food (95) and in the present study the vasodilator effect of a long chain monoglyceride and fatty acid required the presence of a bile salt (Tables 5 and 6). The major aim of this series of experiments

Table 5. Effects of luminal placement of 10 mM taurocholate (TCA) or lipids[+] with and without TCA on local jejunal blood flow and vascular resistance.

	Segment A			Segment B			(N)
	Precontrol	Test	Postcontrol	Precontrol	Test	Postcontrol	
	Saline	TCA	Saline	Saline	Saline	Saline	(6)
Blood Flow	63.1 ± 2.7	62.8 ± 3.1	60.8 ± 3.1	65.9 ± 4.0	65.0 ± 3.6	62.6 ± 4.9	
Resistance	2.4 ± 0.1	2.4 ± 0.2	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	
	Saline	20-MO + TCA	Saline	Saline	20-MO	Saline	(6)
Blood Flow	48.1 ± 8.2	52.9 ± 9.2*	48.9 ± 8.2	46.1 ± 7.1	45.2 ± 7.1	44.4 ± 6.8	
Resistance	3.1 ± 0.5	2.8 ± 0.4*	3.0 ± 0.4	3.2 ± 0.4	3.2 ± 0.4	3.2 ± 0.4	
	Saline	40-0 + TCA	Saline	Saline	40-0	Saline	(6)
Blood Flow	53.9 ± 4.8	64.6 ± 7.0*	57.0 ± 5.4	61.5 ± 8.6	60.9 ± 8.9	60.3 ± 7.4	
Resistance	2.6 ± 0.3	2.3 ± 0.3*	2.6 ± 0.3	2.5 ± 0.4	2.5 ± 0.4	2.5 ± 0.4	
	Saline	20-C + TCA	Saline	Saline	20-C	Saline	(6)
Blood Flow	45.5 ± 3.0	45.1 ± 3.0	46.2 ± 3.7	48.1 ± 3.9	46.2 ± 3.8	46.7 ± 4.2	
Resistance	3.3 ± 0.3	3.3 ± 0.3	3.2 ± 0.3	3.2 ± 0.4	3.3 ± 0.3	3.2 ± 0.3	

Values are means ± S.E. Blood flow = ml/min/100 gm. Resistance = mm Hg/ml/min/100 gm.

+ Lipids: 40-0 = 40 mM oleic acid, 20-MO = 20 mM monoolein, 20-C = 20 mM caproic acid.

* Value is significantly different from precontrol value of each sement ($p < 0.05$, paired Student's t-test).

Table 6. Percent changes from precontrol values of blood flow and vascular resistance of two jejunal segments after luminal placement of 10 mM taurocholate (TCA) or lipids[+] with and without TCA.

	Segment A	Segment B	D = A - B[++]	(N)
	TCA	Saline		(6)
Blood Flow	- 0.7 ± 1.4	- 1.2 ± 1.6	0.5 ± 0.4	
Resistance	- 0.8 ± 1.8	- 0.4 ± 2.0	0.4 ± 0.5	
	20-M0 + TCA	20-M0		(6)
Blood Flow	+ 9.6 ± 1.1*	- 2.0 ± 2.2	11.6 ± 2.5*	
Resistance	- 9.1 ± 0.9*	+ 1.9 ± 2.1	11.0 ± 2.5*	
	40-0 + TCA	40-0		(6)
Blood Flow	+18.7 ± 3.2*	- 1.6 ± 2.0	20.3 ± 3.7*	
Resistance	-14.7 ± 2.4*	+ 2.5 ± 1.9	17.2 ± 3.0*	
	20-C + TCA	20-C		(6)
Blood Flow	- 0.2 ± 4.7	- 3.1 ± 5.2	2.9 ± 1.7	
Resistance	+ 1.8 ± 4.3	+ 3.8 ± 4.2	2.0 ± 1.9	

Values are means ± S.E.

+ Lipids: 40-0 = 40 mM oleic acid. 20-M0 = 20 mM monoolein. 20-C = 20 mM caproic acid.

++ D = A - B represents the difference in the changes occurring simultaneously in the two segments.

* Value is significant ($p < 0.05$, paired Student's t-test).

was to determine if bile, a physiological detergent, would alter the vascular effects of fatty acids, a mixture of 16 amino acids, or glucose. Two concentrations of bile (33 and 10%) were used to cover the range of bile concentration in the upper small intestine after a meal.

The results obtained with 33% bile are shown in Tables 7 and 8. As shown, placement of bile alone in the lumen did not significantly alter flow or resistance. Placement of oleic acid (a long chain fatty acid) or caproic acid (a short chain fatty acid) did not alter flow or resistance unless bile was added to these fatty acids. In the presence of 33% bile both oleic acid and caproic acid increased flow and decreased resistance. When the vascular effects of a 20 mM oleic acid-bile mixture and 20 mM caproic acid-bile mixture were compared, oleic acid increased flow by 24.0% and decreased resistance by 19.9% while caproic acid increased flow by only 11.2% and decreased resistance by 11.1% (Table 8). The difference in the vascular effects of these two solutions (12.8% for blood flow and 8.8% for resistance) was significant (Table 8, D = A - B), indicating that in the presence of bile, the long chain fatty acid (oleic) produced twice as great a hyperemia as did the short chain fatty acid (caproic) on a molar basis. Also shown in Tables 7 and 8 is a comparison of the vascular effects of 40 and 20 mM oleic acid in bile. The 40 mM oleic acid-bile mixture increased flow by 45.6% and decreased resistance by 29.9% while the 20 mM oleic acid-bile mixture increased flow by 20.8% and decreased resistance by 16.9%. The difference in the vascular effects of these two solutions (24.9% for flow and 13.0% for resistance) was significant, indicating that doubling the concentration of the fatty acid in the bile mixture doubled its hyperemic effect.

Table 7. Effects of luminal placement of lipids+, 200 mM glucose, and a 25.2 mM mixture of 16 amino acids (16 AA)+, each with and without 33% bile, on local jejunal blood flow and vascular resistance.

	Segment A			Segment B			(N)
	Precontrol	Test	Postcontrol	Precontrol	Test	Postcontrol	
	Saline	Bile	Saline	Saline	Saline	Saline	(10)
Blood Flow	67.1 ± 8.8	66.5 ± 8.3	63.8 ± 8.2	62.7 ± 8.6	61.0 ± 8.0	60.0 ± 7.5	
Resistance	2.5 ± 0.5	2.5 ± 0.4	2.6 ± 0.4	2.8 ± 0.5	2.8 ± 0.5	2.8 ± 0.5	
	Saline	20-0 + Bile	Saline	Saline	20-0	Saline	(6)
Blood Flow	50.3 ± 4.2	59.7 ± 4.3*	52.1 ± 2.9	54.1 ± 4.9	53.1 ± 4.4	51.0 ± 3.7	
Resistance	2.8 ± 0.2	2.3 ± 0.1*	2.6 ± 0.1	2.6 ± 0.2	2.6 ± 0.2	2.7 ± 0.2	
	Saline	20-C + Bile	Saline	Saline	20-C	Saline	(6)
Blood Flow	60.9 ± 6.3	68.7 ± 6.6*	62.0 ± 6.3	60.2 ± 5.7	60.2 ± 5.5	60.2 ± 5.7	
Resistance	2.4 ± 0.3	2.0 ± 0.2*	2.3 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	
	Saline	20-0 + Bile	Saline	Saline	20-C + Bile	Saline	(6)
Blood Flow	42.4 ± 4.4	52.2 ± 5.6*	43.8 ± 5.0	41.9 ± 5.1	46.4 ± 5.3*	42.5 ± 5.8	
Resistance	3.4 ± 0.3	2.7 ± 0.2*	3.3 ± 0.3	3.5 ± 0.4	3.1 ± 0.3*	3.5 ± 0.5	
	Saline	40-0 + Bile	Saline	Saline	20-0 + Bile	Saline	(6)
Blood Flow	50.8 ± 4.5	74.6 ± 9.1*	51.3 ± 6.7	48.9 ± 6.4	58.6 ± 7.4*	46.5 ± 5.8	
Resistance	2.8 ± 0.2	2.0 ± 0.2*	3.0 ± 0.4	3.1 ± 0.3	2.5 ± 0.2*	3.4 ± 0.5	
	Saline	16 AA + Bile	Saline	Saline	16 AA	Saline	(5)
Blood Flow	42.0 ± 4.5	47.0 ± 4.8*	45.8 ± 4.9	42.3 ± 4.1	41.7 ± 4.1	42.6 ± 4.4	
Resistance	3.6 ± 0.4	3.1 ± 0.3*	3.3 ± 0.3	3.5 ± 0.3	3.5 ± 0.3	3.5 ± 0.4	
	Saline	Glucose + Bile	Saline	Saline	Glucose	Saline	(9)
Blood Flow	51.3 ± 4.5	62.3 ± 5.1*	53.8 ± 5.1	51.4 ± 4.3	54.2 ± 4.2*	48.1 ± 3.8	
Resistance	3.0 ± 0.3	2.5 ± 0.3*	2.9 ± 0.4	3.0 ± 0.3	2.8 ± 0.3*	3.1 ± 0.3	

Values are means ± S.E. Blood Flow = ml/min/100 gm. Resistance = mm Hg/ml/min/100 gm.

+ Lipids: 20-0 = 20 mM oleic acid, 40-0 = 40 mM oleic acid, 20-C = 20 mM caproic acid.
16 AA: see Table 1 for constituent amino acids and their concentrations.

* Value is significantly different from the precontrol value of each segment ($p < 0.05$, paired Student's t-test).

Table 8. Percent change from precontrol values of blood flow and vascular resistance of two jejunal segments after luminal placement of lipids[+], 200 mM glucose, and a 25.2 mM mixture of 16 amino acids (16 AA)[+], each with and without 33% bile.

	Segment A	Segment B	D = A-B[++]	(N)
	Bile	Saline		(10)
Blood Flow	+ 0.6 ± 2.9	- 1.3 ± 2.8	1.8 ± 2.2	
Resistance	+ 0.9 ± 2.9	+ 2.7 ± 3.3	1.8 ± 2.0	
	20-0 + Bile	20-0		(6)
Blood Flow	+19.5 ± 3.2*	- 1.3 ± 1.5	20.8 ± 3.5*	
Resistance	-14.8 ± 2.2*	+ 2.7 ± 2.2	17.4 ± 3.1*	
	20-C + Bile	20-C		(6)
Blood Flow	+13.3 ± 3.1*	+ 0.1 ± 1.8	13.2 ± 3.3*	
Resistance	-13.5 ± 2.5*	- 2.3 ± 2.1	11.2 ± 2.7*	
	20-0 + Bile	20-C + Bile		(6)
Blood Flow	+24.0 ± 3.8*	+11.2 ± 1.8*	12.8 ± 3.0*	
Resistance	-19.9 ± 3.4*	-11.1 ± 2.0*	8.8 ± 1.8*	
	40-0 + Bile	20-0 + Bile		(6)
Blood Flow	+45.6 ± 9.9*	+20.8 ± 5.5*	24.9 ± 8.1*	
Resistance	-29.9 ± 5.4*	-16.9 ± 3.6*	13.0 ± 4.0*	
	16 AA + Bile	16 AA		(5)
Blood Flow	+12.4 ± 3.9*	- 1.3 ± 2.5	13.7 ± 2.2*	
Resistance	-11.7 ± 2.6*	+ 0.3 ± 2.3	12.1 ± 1.8*	
	Glucose + Bile	Glucose		(9)
Blood Flow	+21.9 ± 2.9*	+ 6.0 ± 1.5*	15.9 ± 2.2*	
Resistance	-18.1 ± 2.2*	- 6.3 ± 1.4*	11.8 ± 1.5*	

Values are means ± S.E.

\+ Lipids: 20-0 = 20 mM oleic acid, 40-0 = 40 mM oleic acid, 20-C = 20 mM caproic acid.
16 AA: see Table 1 for constituent amino acids and their concentrations.

++ D = A-B represents the difference in changes occurring in the two segments.

* Value is significant ($p < 0.05$, student's t-test).

The effect of 33% bile on the vasoactivity of glucose and a mixture of 16 amino acids is also shown in Tables 7 and 8. The 25.2 mM mixture of 16 amino acids did not significantly alter local blood flow or vascular resistance, but addition of bile to the 16 amino acids produced a significant increase in flow and decrease in resistance. A mixture of glucose and bile produced a greater increase in flow and decrease in resistance than did glucose alone. The glucose-bile mixture increased flow by 21.9% and decreased resistance by 18.1% while glucose alone increased flow by 6.0% and decreased resistance by 6.3%. The difference in the vascular changes produced by these two solutions (15.9% for flow and 11.8% for resistance) was significant (Table 8, D = A - B), indicating that the mixture of glucose and bile had three times the hyperemic effect as glucose alone had.

The effects of 10% bile on the vasoactivity of glucose, amino acids, and fatty acids are shown in Tables 9 and 10. In some cases the results obtained with 10% bile were similar to those obtained with 33% bile. As shown in Tables 9 and 10, luminal placement of 10% bile did not alter jejunal blood flow or vascular resistance. Again, oleic acid did not alter flow or resistance unless bile was added to the fatty acid. In the presence of bile, oleic acid increased flow by 24.0% and decreased resistance by 18.1%. Also, a glucose-bile mixture produced a greater increase in flow and decrease in resistance than did glucose alone. The glucose-bile mixture increased flow by 9.9% and decreased resistance by 8.3% while glucose alone increased flow by 4.8% and decreased resistance by 3.8%. The difference in the vascular effects of these two solutions was significant, indicating that the mixture of glucose and bile had twice the hyperemic effect as glucose alone had.

Table 9. The effects of luminal placement of 20 mM oleic acid (20-0), 20 mM caproic acid (20-C), 200 mM glucose, a 25.2 mM mixture of 16 amino acids (16 AA)+, or a 37.5 mM mixture of 3 dipeptides (3 DP)+, each with and without 10% bile, on local jejunal blood flow and vascular resistance.

	Segment A			Segment B			
	Precontrol	Test	Postcontrol	Precontrol	Test	Postcontrol	
	Saline	Bile	Saline	Saline	Saline	Saline	(7)
Blood Flow	53.6 ± 6.6	54.8 ± 6.5	55.4 ± 6.0	51.9 ± 6.3	51.2 ± 6.1	54.7 ± 6.0	
Resistance	2.6 ± 0.3	2.5 ± 0.3	2.5 ± 0.3	2.7 ± 0.4	2.7 ± 0.4	2.6 ± 0.4	
	Saline	20-0 + Bile	Saline	Saline	20-0	Saline	(8)
Blood Flow	45.2 ± 3.4	55.6 ± 4.0*	44.3 ± 3.2	45.5 ± 3.2	43.3 ± 2.7	42.7 ± 2.1	
Resistance	3.0 ± 0.2	2.4 ± 0.2*	3.1 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	3.2 ± 0.2	
	Saline	20-C + Bile	Saline	Saline	20-C	Saline	(8)
Blood Flow	42.1 ± 3.6	41.0 ± 3.4	39.4 ± 3.3	37.2 ± 2.0	37.7 ± 3.1	38.2 ± 2.4	
Resistance	3.3 ± 0.4	3.4 ± 0.4	3.5 ± 0.4	3.6 ± 0.2	3.6 ± 0.4	3.5 ± 0.2	
	Saline	Glucose + Bile	Saline	Saline	Glucose	Saline	(10)
Blood Flow	44.8 ± 2.5	48.9 ± 2.5*	43.0 ± 2.8	42.6 ± 1.5	44.6 ± 1.5*	39.2 ± 1.6	
Resistance	3.0 ± 0.2	2.8 ± 0.2*	3.2 ± 0.3	3.1 ± 0.2	3.0 ± 0.1*	3.4 ± 0.2	
	Saline	16 AA + Bile	Saline	Saline	16 AA	Saline	(8)
Blood Flow	51.0 ± 5.3	50.7 ± 5.3	51.4 ± 5.4	47.4 ± 5.1	46.2 ± 4.5	48.0 ± 4.6	
Resistance	2.7 ± 0.4	2.8 ± 0.4	2.8 ± 0.5	2.9 ± 0.3	2.9 ± 0.3	2.9 ± 0.3	
	Saline	3 DP + Bile	Saline	Saline	3 DP	Saline	(8)
Blood Flow	44.5 ± 5.3	45.2 ± 5.7	42.7 ± 5.3	46.4 ± 6.9	45.9 ± 7.7	43.8 ± 5.8	
Resistance	3.4 ± 0.3	3.3 ± 0.3	3.5 ± 0.4	3.3 ± 0.4	3.4 ± 0.4	3.5 ± 0.4	

Values are means ± S.E. Blood Flow = ml/min/100 gm. Resistance = mm Hg/ml/min/100 gm.

+ 16AA and 3 DP: see Table 1 for constituent amino acids and peptides and their concentrations.

* Value is significantly different from the precontrol value of each segment ($p < 0.05$, paired Student's t-test).

Table 10. Percent change from precontrol values of blood flow and vascular resistance of two jejunal segments after luminal placement of 20 mM oleic acid (20-0), 20 mM caproic acid (20-C), 200 mM glucose, a 25.2 mM mixture of 16 amino acids (16 AA)$^{+}$, or a 37.5 mM mixture of 3 dipeptides (3 DP)$^{+}$, each with and without 10% bile.

	Segment A	Segment B	D = A-B^{++}	(N)
	Bile	Saline		(7)
Blood Flow	+ 2.7 ± 3.0	-1.0 ± 2.8	3.7 ± 4.0	
Resistance	- 3.0 ± 2.8	0.6 + 3.2	3.6 ± 3.5	
	20-0 + Bile	20-0		(8)
Blood Flow	+24.0 ± 6.8*	-4.2 ± 2.4	28.2 ± 6.1*	
Resistance	-18.1 ± 3.7*	+5.2 ± 2.6	23.3 ± 3.4*	
	20-C + Bile	20-C		(8)
Blood Flow	- 1.6 ± 3.5	0.8 ± 4.7	2.4 ± 2.8	
Resistance	+ 2.0 ± 4.0	-0.1 ± 4.2	2.1 ± 2.7	
	Glucose + Bile	Glucose		(10)
Blood Flow	+ 9.9 ± 2.0*	+4.8 ± 1.8*	5.1 ± 1.4*	
Resistance	- 8.3 ± 1.4*	-3.8 ± 1.3*	4.5 ± 1.3*	
	16 AA + Bile	16 AA		(8)
Blood Flow	- 0.3 ± 3.8	-1.3 ± 3.0	1.0 ± 2.1	
Resistance	+ 1.4 ± 3.8	+1.8 ± 2.8	0.4 ± 2.3	
	3 DP + Bile	3 DP		(8)
Blood Flow	+ 1.4 ± 3.0	- 2.2 ± 2.2	3.6 ± 3.3	
Resistance	- 2.8 ± 2.6	+ 0.7 ± 2.3	3.5 ± 3.2	

Values are means and S.E.

+ 16 AA and 3 DP: see Table 1 for constituent amino acids and peptides and their concentrations.

++ D = A-B represents the difference in changes occurring simultaneously in the two segments.

* Value is significant ($p < 0.05$, paired Student's t-test).

The effects of 10% bile on the vasoactivities of caproic acid and a mixture of 16 amino acids was different from those obtained with 33% bile. Caproic acid did not alter flow or vascular resistance whether it was placed in the lumen alone or with 10% bile. Similarly, the 25.2 mM mixture of 16 amino acids did not alter flow or resistance whether it was placed in the lumen with or without 10% bile.

Some amino acids are absorbed in dipeptide form rather than as free amino acids, and thus the vascular effects of a 37.5 mM mixture of 3 dipeptides (gly-asp, gly-glu, and pro-leu) were studied with and without 10% bile. As shown in Tables 9 and 10, the mixture of dipeptides did not alter flow or resistance whether it was placed in the lumen with or without 10% bile.

Others have shown that intraduodenal triglycerides (corn oil) can increase intestinal blood flow (38). To determine whether triglycerides must be digested before the hyperemic response to lipids occurs, additional experiments were performed and the results are shown in Table 11. A hyperemic response occurred only when both bile and pancreatic enzymes were added to triolein. Triolein alone, with bile or with pancreatic enzymes did not alter flow or resistance.

Series 3

The results of the previous series of experiments indicate that only the vasoactivities of glucose and oleic acid were altered by both concentrations of bile. The aim of this series of experiments was to

Table 11. Effects of luminal placement of 10 mM triolein (TriO), triolein with pancreatic enzymes (PE), triolein with 10% bile, and triolein with pancreatic enzymes and 10% bile on local jejunal blood flow and vascular resistance (N = 5).

	Segment A			Segment B			D = A-B+
	Precontrol	Test	Postcontrol	Precontrol	Test	Postcontrol	
	Saline	TriO + Bile	Saline	Saline	TriO	Saline	
Blood Flow	53.9 ± 6.2	50.8 ± 4.6	49.5 ± 4.9	50.4 ± 8.8	46.3 ± 6.4	44.9 ± 5.5	
%	-4.3 ± 4.7			- 5.6 ± 4.5			1.3 ± 2.4
Resistance	2.5 ± 0.1	2.7 ± 0.2	2.8 ± 0.1	2.9 ± 0.4	3.0 ± 0.3	3.1 ± 0.3	
%	+4.9 ± 4.8			+ 6.2 ± 3.6			1.3 ± 3.0
	Saline	TriO + PE	Saline	Saline	TriO + PE + Bile	Saline	
Blood Flow	46.0 ± 3.9	45.0 ± 2.7	42.4 ± 2.0	48.3 ± 3.3	54.1 ± 3.0*	46.4 ± 3.1	
%	-1.2 ± 2.7			+12.5 ± 2.4**			13.7 ± 0.8**
Resistance	2.9 ± 0.2	3.0 ± 0.2	3.1 ± 0.1	2.8 ± 0.2	2.5 ± 0.2*	2.9 ± 0.2	
%	+1.5 ± 3.0			-11.1 ± 2.0**			12.6 ± 1.3**

Values are means ± S.E. Blood Flow = ml/min/100 gm. Resistance = mm Hg/ml/min/100 gm.

+ D = A-B represents the difference in changes occurring simultaneously in the two segments.

* Value is significantly different from the precontrol value of each segment ($p < 0.05$, paired Student's t-test).

** Value is significant ($p < 0.05$, paired student's t-test).

determine whether bile can alter the absorption rates of these two nutrients.

Glucose Absorption. The effect of 33% bile on glucose absorption is shown in Table 12. Placement of glucose alone or glucose with bile increased jejunal blood flow (glucose by 4.2 ml/min/100 gm and glucose with bile by 8.7 ml/min/100 gm). The hyperemic effects of these two glucose solutions were significantly different (Table 12, D = A - B) indicating that bile enhanced the hyperemic effect of glucose. Both glucose solutions increased the venous glucose concentration resulting in an increase in V-A glucose difference. However, the V-A glucose difference was greater for the solution containing glucose alone than that for the solution containing glucose and bile (Table 12, D = A - B). The increase in glucose absorption (V-A glucose difference x blood flow) produced by glucose alone was therefore not significantly different from that produced by the glucose-bile mixture (Table 12, D = A - B), indicating that bile did not influence glucose absorption.

The oxygen consumption of the two segments were also compared. As shown in Table 13, addition of 33% bile to glucose produced a significantly greater hyperemia than did glucose alone (Table 13, D = A - B). Both glucose solutions increased the A-V oxygen difference. However, the A-V oxygen difference was greater for the solution containing glucose alone than for the solution containing glucose and bile (Table 13, D = A - B). The increase in oxygen consumption produced by glucose alone was not significantly different from that produced by the glucose-bile mixture (Table 13, D = A - B), indicating that bile did not influence the increase in oxygen consumption which occurred while glucose was in the lumen.

Table 12. Effects of luminal placement of 200 mM glucose in one segment and 200 mM glucose - 33% bile mixture in the other segment on blood flow (ml/min/100 gm), V-A glucose difference (mg/100 ml), and glucose absorption (mg/min/100 gm) of two jejunal segments (N = 8).

	Segment A			Segment B			D = A-B+
	Saline	Glucose + Bile	Saline	Saline	Glucose	Saline	
Blood Flow	49.4 ± 4.5	58.1* ± 4.8	51.5 ± 5.1	48.1 ± 4.0	52.3* ± 4.2	45.3 ± 4.0	
Δ		+ 8.7 ± 1.3**			+ 4.2 ± 0.5**		4.5 ± 1.2**
V-A Glucose	- 6.5 ± 5.4	63.5* ± 8.7	- 1.4 ± 3.6	- 3.9 ± 4.1	82.9* ±11.8	- 6.7 ± 9.1	
Δ		+ 69.9 ± 11.2**			86.9 ± 12.6**		16.9 ± 6.8**
Glucose Absorption	- 3.2 ± 2.4	36.7* ± 5.6	- 0.7 ± 1.9	- 2.4 ± 2.0	41.9* ± 5.1	- 3.4 ± 4.0	
Δ		+39.9 ± 6.0**			+44.4 ± 5.9**		4.4 ± 4.5

Values are means ± S.E. of those measurements made 15 minutes after the placement of saline and the average of three measurements made 7, 11, and 15 minutes after the placement of glucose solutions.

Δ = Changes from precontrol values.

+ D = A-B represents the difference in changes (Δ) occurring simultaneously in the two segments.

* Value is significantly different from the precontrol value of each segment ($p < 0.05$, paired Student's t-test).

** Value is significant ($p < 0.05$, paired Student's t-test).

Table 13. Effects of luminal placement of 200 mM glucose in one segment and 200 mM glucose - 33% bile mixture in the other segment on blood flow (ml/min/100 gm), A-V oxygen difference (ml/100 ml), and oxygen consumption (ml/min/100 gm) of two jejunal segments (N = 6).

	Segment A			Segment B			D = A-B[+]
	Saline	Glucose + Bile	Saline	Saline	Glucose	Saline	
Blood Flow	42.6 ± 4.5	50.0* ± 4.7	44.0 ± 4.6	41.2 ± 4.5	43.8* ± 4.2	39.6 ± 4.1	
Δ	+ 7.4 ± 1.1**			+ 2.6 ± 0.6**			4.8 ± 1.2**
A-V Oxygen	5.2 ± 0.4	5.5* ± 0.4	5.0 ± 0.5	5.7 ± 0.5	6.4* ± 0.4	5.7 ± 0.5	
Δ	+ 0.3 ± 0.1**			+ 0.7 ± 0.2**			0.4 ± 0.1**
Oxygen Consumption	2.2 ± 0.2	2.7* ± 0.2	2.1 ± 0.1	2.3 ± 0.3	2.7* ± 0.3	2.3 ± 0.2	
Δ	+ 0.5 ± 0.1**			+ 0.4 ± 0.1**			0.1 ± 0.1

Values (means ± S.E.) shown are those of one measurement made while saline was in the lumen or the average of two measurements made while glucose solutions were in the lumen.

Δ = changes from precontrol values.

+ D = A-B represents the difference in the changes (Δ) occurring simultaneously in the two segments.

* Value is significantly different from the precontrol value of each segment ($p < 0.05$, paired Student's t-test).

** Value is significant ($p < 0.05$, paired Student's t-test).

Simultaneous measurements of blood flow, glucose absorption and oxygen consumption were obtained in five animals (Figure 3). Bile enhanced the hyperemic effect of glucose but did not alter glucose absorption or oxygen consumption.

The effects of 10% bile on glucose absorption were also studied and the results are shown in Table 14. Bile enhanced the hyperemic effect of glucose but did not alter glucose absorption.

Oleic Acid Absorption. The effects of 10% bile on the vasoactivity and absorption of oleic acid are shown in Figure 4. Oleic acid produced a hyperemia only in the presence of bile. Oleic acid was absorbed from the lumen (Figure 4, middle panel) and entered the mucosal tissue (Figure 4, lower panel) whether bile was present or not. However, in the presence of bile more oleic acid was absorbed from the lumen.

Different results were obtained when the pH of the two oleic acid solutions was raised to 9.5 (Figure 5). Oleic acid increased blood flow to the same extent with or without bile, but oleic acid was absorbed to a greater extent with bile than without. Placement of normal saline at pH 9.5 did not alter jejunal blood flow from the control (normal saline at pH 7.0) values.

Table 14. Effects of luminal placement of 200 mM glucose in one segment and 200 mM glucose - 10% bile mixture in the other segment on local blood flow (ml/min/100 gm), V-A glucose difference (mg/100 ml) and glucose absorption (mg/min/100 gm) of two jejunal segments (N = 9).

	Segment A			Segment B			D = A-B+
	Saline	Glucose + Bile	Saline	Saline	Glucose	Saline	
Blood Flow	46.6 ± 3.0	50.8* ± 2.8	45.1 ± 3.6	42.2 ± 2.4	44.7* ± 2.1	38.8 ± 2.4	
Δ	+ 4.2 ± 1.0**			+ 2.5 ± 0.9**			1.7 ± 0.6**
V-A Glucose	- 0.6 ± 4.4	+88.9 ±23.7	- 2.6 ± 2.7	- 9.5 ± 3.6	+96.3 ±21.2	- 1.6 ± 3.3	
Δ	+ 88.6 ± 24.5			+105.8 ± 23.3			17.2 ± 12.8
Glucose Absorption	- 0.1 ± 1.9	42.4 ± 8.2	- 1.3 ± 1.2	- 4.1 ± 1.7	41.8 ± 8.0	- 0.3 ± 1.2	
Δ	+ 42.2 ± 8.4			+ 45.9 ± 8.9			3.7 ± 5.3

Values are means ± S.E. of those measurements made 15 minutes after placement of saline and the average of three measurements made 7, 11, and 15 minutes after the placement of glucose solution.

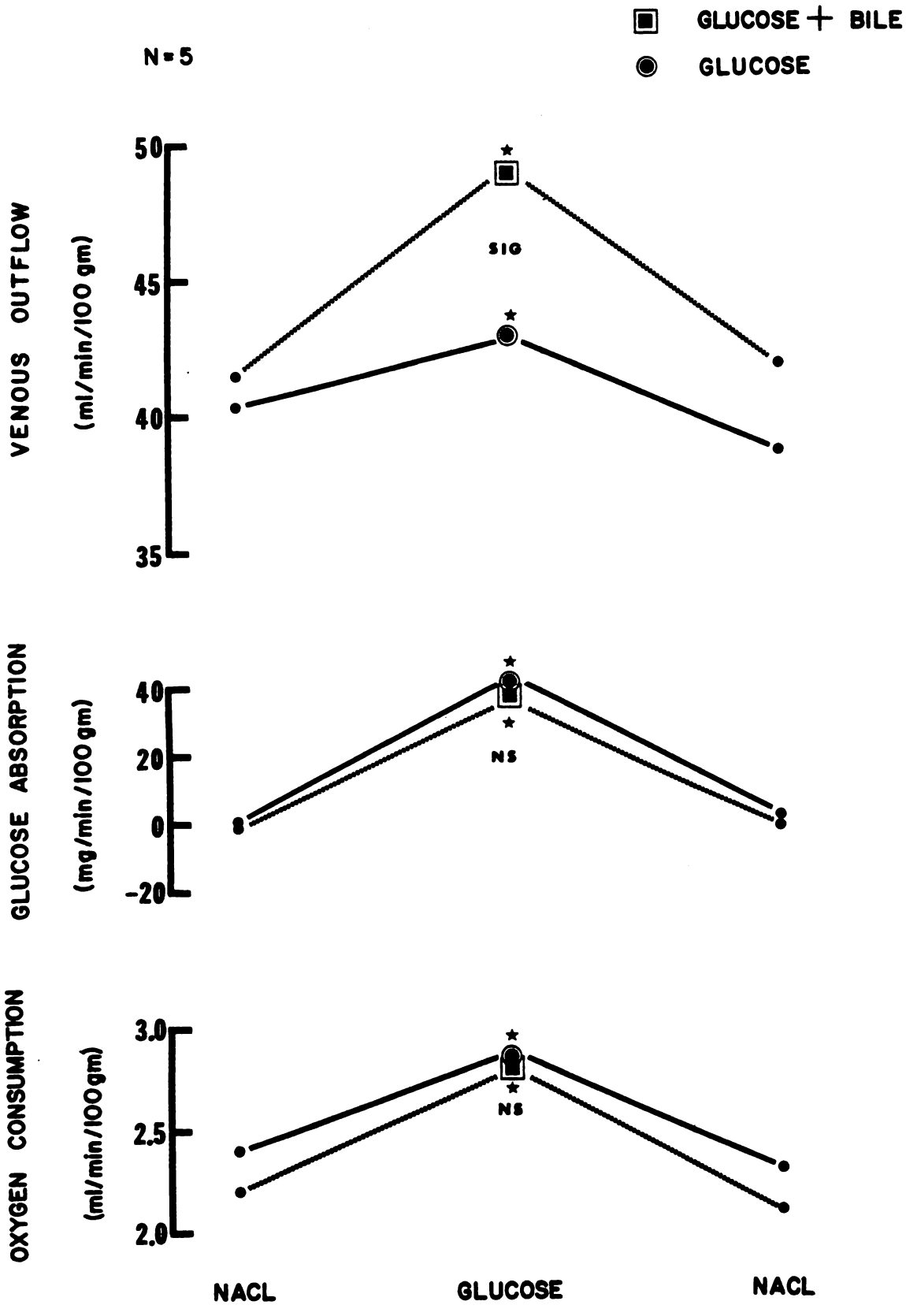
Δ = Change from precontrol values.

+ D = A-B represents the difference in changes (Δ) occurring simultaneously in the two segments.

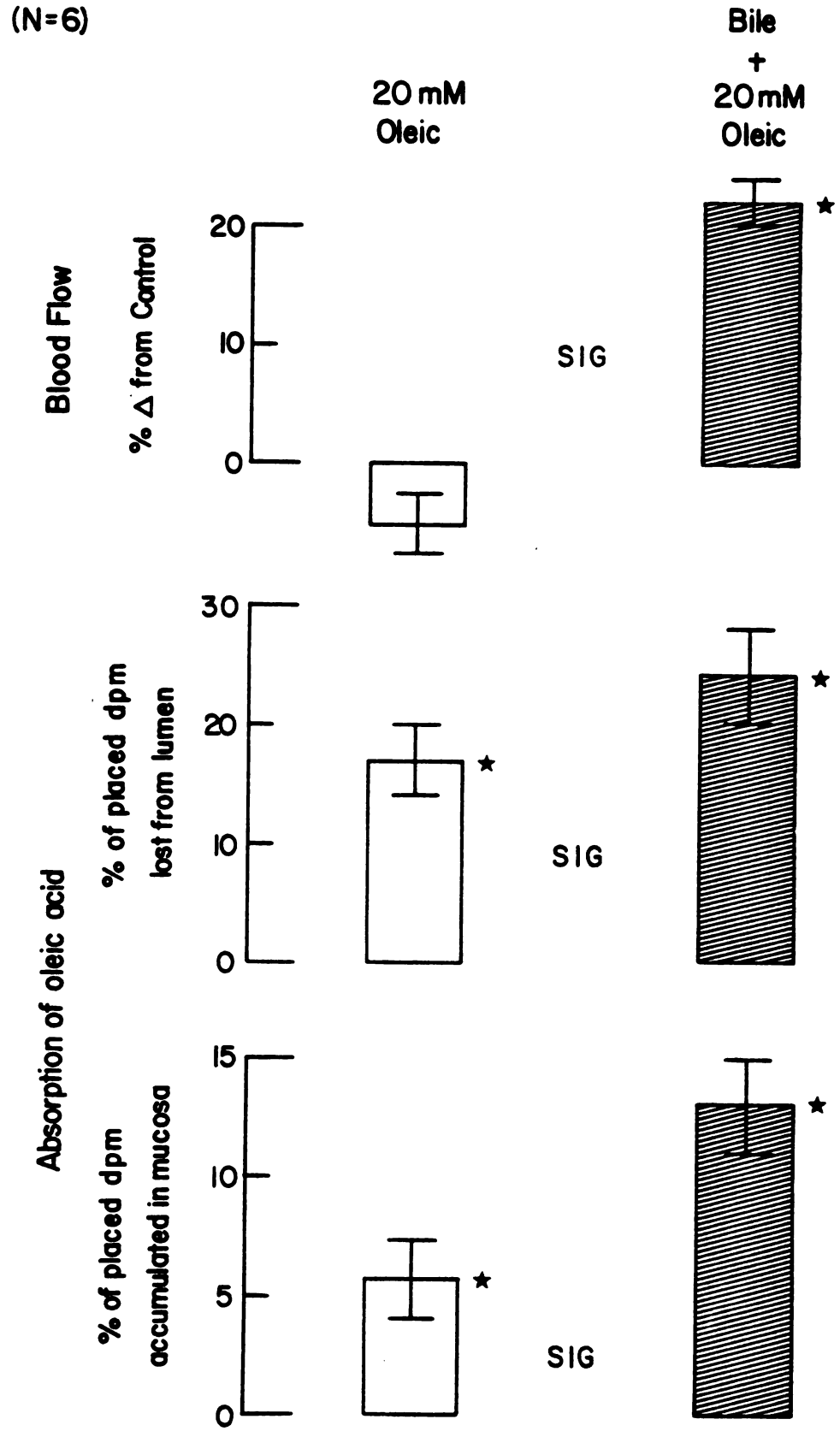
* Value is significantly different from the precontrol value of each segment ($p < 0.05$, paired student's t-test).

** Value is significant ($p < 0.05$, paired student's t-test).

3. Comparison of the effects of luminal placement of 200 mM glucose in one segment and a 200 mM glucose - 33% bile mixture in the other segment on blood flow, glucose absorption, and oxygen consumption of two jejunal segments.

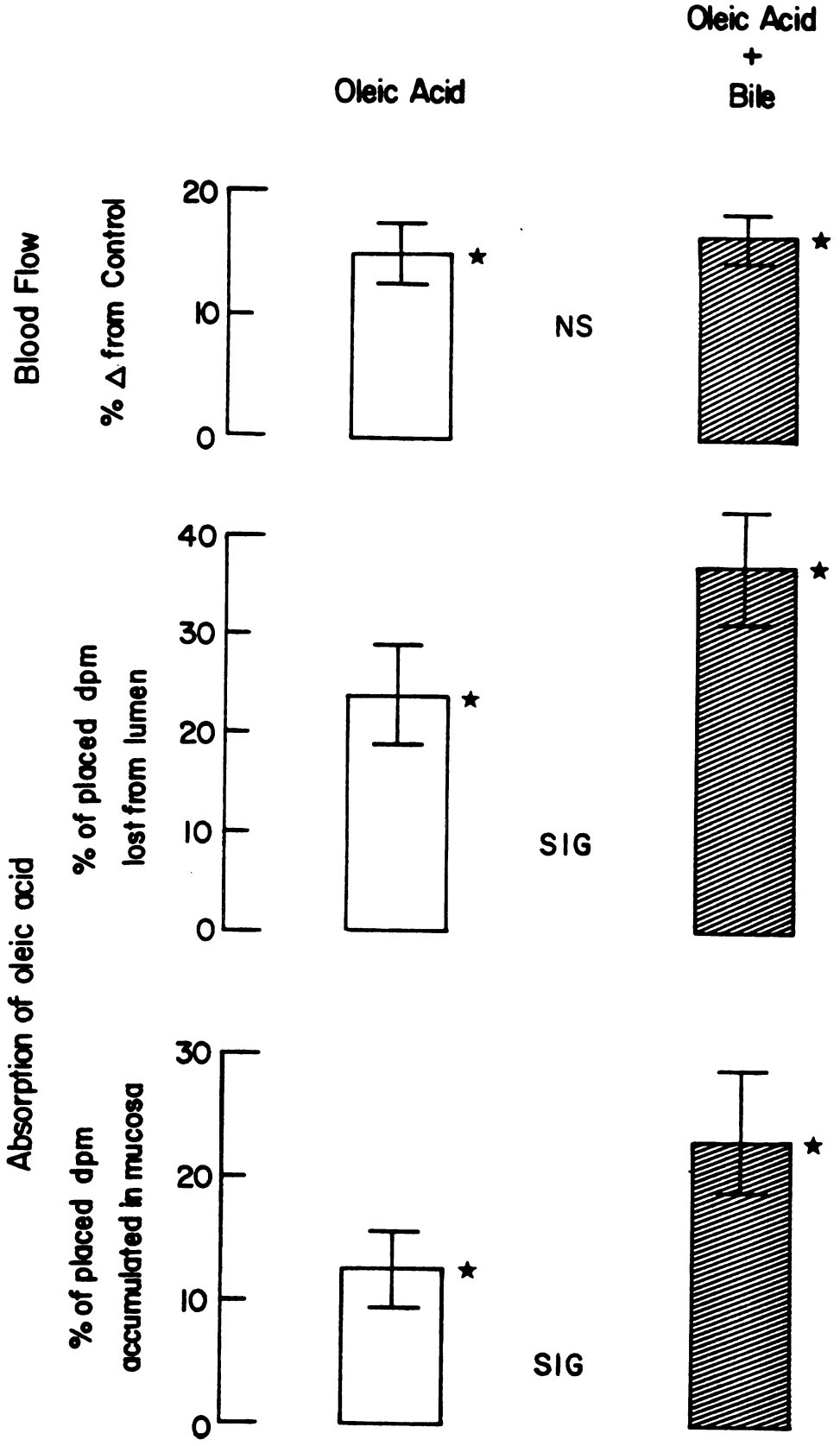

GLUCOSE + BILE
GLUCOSE
N=5
VENOUS OUTFLOW
(ml/min/100 gm)
50
45
40
35
SIG
GLUCOSE ABSORPTION
(mg/min/100 gm)
40
20
0
-20
NS
OXYGEN CONSUMPTION
(ml/min/100gm)
3.0
2.5
2.0
NS
NACL
GLUCOSE
NACL

4. Comparison of the effects of luminal placement of 200 mM oleic acid (pH 7.0) in one segment and 20 mM oleic acid - bile mixture (pH 7.0) in the other segment on blood flow and oleic acid absorption.


pH=7.0
(N=6)
20 mM Oleic
Bile + 20mM Oleic
Blood Flow
% Δ from Control
20
10
0
SIG
Absorption of oleic acid
% of placed dpm lost from lumen
30
20
10
0
SIG
% of placed dpm accumulated in mucosa
15
10
5
0
SIG

5. Comparison of the effects of luminal placement of 20 mM oleic acid (pH 9.5) in one segment and 20 mM oleic acid - 10% bile mixture (pH 9.5) in the other segment on blood flow and oleic acid absorption.

pH = 9.5
(N = 7)


Oleic Acid
Oleic Acid + Bile
Blood Flow
% Δ from Control
20
10
0
NS
Absorption of oleic acid
% of placed dpm lost from lumen
40
30
20
10
0
SIG
% of placed dpm accumulated in mucosa
30
20
10
0
SIG

CHAPTER V

DISCUSSION

Blood flow to the small intestine increases by as much as 200% after a meal (18, 41, 42, 43, 84, 103, 104, 105), and the hyperemia has been shown to be confined to that portion of the small intestine which is exposed to chyme (26, 70). The chyme of the upper small intestine consists of undigested food, hydrolytic products of food digestion, pancreatic enzymes and bile. Previous studies have shown that food, digested in vitro with pancreatic enzymes, increases local jejunal blood flow when placed in the lumen (26, 70, 87, 95). In contrast, intrajejunal placement of undigested food, pancreatic enzymes or bile does not alter local blood flow (70, 95). These findings indicate that the substances responsible for the postprandial intestinal hyperemia are likely to be the hydrolytic products of food digestion. This contention is supported by the finding that a solution containing glucose, amino acids, and micellar fatty acid and monoglyceride (all at postprandial intrajejunal concentrations) increased jejunal blood flow (Figure 2 and Table 2). Furthermore, the magnitude of the hyperemia produced by this mixture (+38.2%) was comparable to that produced by digested food (+10 - 40%) in a similar preparation of the jejunum (26, 70, 95). The next step of the present study was to determine which constituents of this mixture were responsible for the hyperemia.

Glucose, the major end-product of carbohydrate digestion, has been shown to be present in the jejunal lumen in concentrations ranging from 28

to 222 mM after meals (14). Placement of a 150 - 200 mM glucose solution increased local jejunal blood flow by 4.8 - 8.7 percent (Tables 8, 10, 12, 13 and 14). Qualitatively and quantitatively, this response is similar to those reported by investigators using a similar intestinal preparation and method of measuring blood flow (21, 87, 102). Thus, there is evidence to support a role for glucose in the postprandial intestinal hyperemia.

Other workers (22, 38, 101) using a different preparation of the duodenum or jejunum and/or different method of measuring intestinal blood flow (flow meters on the superior mesenteric artery or ^{133}Xe clearance technique) did not detect any change in flow when 280 mM glucose was introduced into the lumen. Because in the present study glucose (150 - 200 mM) increased jejunal flow by only 4.8 - 8.7%, it is possible that under different experimental conditions this small change in flow may not be detected.

The products of protein digestion are amino acids and peptides of varying chain length (48, 83). The concentration of amino acids in the jejunum after meals has been reported to range from 4 - 29 mM for amino acids in free form (2, 83, 85) and 118 mM for amino acids in peptide form (2). In the present study, intrajejunal placement of a 37.5 mM mixture of 3 dipeptides or a 25.2 mM mixture of 16 amino acids did not alter local blood flow (Tables 2, 7 and 9). These findings indicate that the major hydrolytic products of protein digestion do not contribute to the postprandial intestinal hyperemia.

To determine whether amino acids are vasoactive at greater luminal concentrations, the concentration of each amino acid in the mixture was increased tenfold. As shown in Table 3, a 252 mM mixture of the 16 amino

acids did increase flow when placed into the lumen. Subsequent experiments indicate that of the 16 amino acids in this mixture only 28 mM glutamic acid and 20 mM aspartic acid increased flow, while the other 14 amino acids did not alter flow (Table 3). Moreover, the hyperemic effects of these two amino acids were similar (Table 4). Since the concentrations of these two amino acids were approximately 10 times those found in the gut after meals, further experiments were performed to determine whether these two amino acids could increase flow at 5 times their physiological luminal concentrations. As shown in Table 3 (bottom row), neither 14 mM glutamic acid nor 10 mM aspartic acid significantly altered local blood flow. Thus, these two amino acids increase intestinal blood flow only when present at concentrations between 5 and 10 times greater than those found in the lumen postprandially.

The reason why only glutamic acid and aspartic acid (of the 16 amino acids studied) produced a hyperemia in the present study is not clear. Glutamic acid and aspartic acid are the only two amino acids which are significantly metabolized inside the mucosal cell during their absorption. These two amino acids undergo transamination with pyruvate to form alanine, CO_2 and other metabolites (78, 81, 111, 113). Some of these metabolites may be vasodilators in the intestine and the hyperemic effect of these two amino acids may result from release of vasodilator metabolites during their absorption.

Other investigators have shown that instillation of 127 mM phenylalanine into the cat duodenum increases superior mesenteric artery blood flow (38) and placement of 300 mM glycine into the canine jejunum increases local blood flow (102). In the present study neither phenylalanine (1.3 and

13.0 mM) nor glycine (2.1 and 21.0 mM) produced a hyperemia when placed in the lumen. The difference in results is probably due to the differences in the concentrations used. It is possible that these two amino acids, which in the present study did not elicit a vasodilator response, may have done so if their luminal concentrations had been higher. The total concentrations of free amino acids present in the intestinal lumen after a meal containing 15 g milk protein, 50 g bovine serum albumin or 200 g lean beef have been shown to be 3.9 (83), 29.3 (2), and 17.5 mM (85) respectively. The luminal concentrations of glu, asp, phe and gly were 0.6 - 2.6, 0.2 - 1.4, 0.2 - 1.8 and 0.2 - 0.7 mM, respectively (2, 83, 85). Thus, the hyperemic effect of glutamic acid (28 mM), aspartic acid (20 mM), phenylalanine (127 mM) and glycine (300 mM) observed in the present study and other studies (38, 102) appears to be aphysiological.

The major hydrolytic products of triglyceride digestion are monoglycerides and fatty acids (94). These lipids are generally found in association with bile salts, forming micelles, which increases their solubility in the aqueous lumen contents (62). The postprandial luminal concentration of monoglycerides, fatty acids, and bile salts have been shown to be 0 - 16 mM (59), 4 - 75 mM (59) and 2 - 28 mM (14, 40), respectively. Intrajejunal placement of a mixture of micellar lipids containing 20 mM monoolein, 40 mM oleic and 10 mM taurocholate increased local blood flow (Table 2). The hyperemic effect of this micellar lipid solution requires the presence of the bile salt, taurocholate, and both of the lipids contribute to the response. As shown in Tables 5 and 6, taurocholate, oleic acid or monoolein alone in the lumen did not alter jejunal blood flow, but when taurocholate is added to either lipid, both mixtures significantly increase flow. Thus,

micellar solutions of the hydrolytic products of lipid digestion are involved in the postprandial intestinal hyperemia.

Bile is an important constituent of intestinal chyme. Sit et al. (95) have shown that intrajejunal placement of bile does not alter local blood flow, but addition of this bile to digested food enhances the hyperemic effect of luminal food. The enhancement of the hyperemic effect of digested food by bile may be related to the well known action of the bile salts of bile in facilitating digestion and absorption of lipids (31). This hypothesis is supported by the findings that the lipolytic products, oleic acid and monoolein, require the presence of taurocholate, a bile salt, to produce a hyperemia. However, in addition to lipolytic products, digested food contains hydrolytic products of protein and carbohydrate digestion. Bile could be enhancing the hyperemic effects of digested food by also altering the vascular effects of these latter components of digested food. Thus, the second major aim of the present study was to determine whether bile alters the vascular effects of all or just some of the major hydrolytic products of food digestion.

The exact concentration of bile in the upper small intestine postprandially is not known. Borgstrom et al. (14) intubated human subjects and monitored changes in bile constituents (bile acids and phospholipids) of the intestinal lumen after a meal. During the first half hour after ingestion of a meal the gall bladder contracts producing a high concentration of bile constituents in the intestinal lumen. The concentration of these bile constituents then gradually declines to a lower value by one hour after the meal and remains relatively constant for the duration of the digestive period. The concentrations of luminal bile acids, one of the major

constituents of bile, can rise to 10 - 17 mg/ml within one half hour after a meal corresponding to the evacuation of the gall bladder. The concentration of bile acids then decline to 2 - 5 mg/ml and remains at these levels for the rest of the digestive period. Nakayama and Johnston (80) have shown that the concentration of bile acids in human gall bladder bile is 48.5 mg/ml. From these reported concentrations of bile acids in gall bladder bile and lumen contents postprandially, the dilution factors for the gall bladder bile used in the present study were derived. The higher and lower concentrations of luminal bile postprandially were approximated by diluting gall bladder bile with 2 parts saline (33% bile) and 9 parts saline (10% bile), respectively.

The vascular effects of both the long chain fatty acid, oleic acid, and the short chain fatty acid, caproic acid, were affected by bile (Tables 7 - 10). The effect of bile on caproic acid, but not oleic acid was dependent on the concentration of bile. As shown in Tables 7 - 10, neither fatty acid altered local blood flow when placed in the lumen without bile. In 10% bile, oleic acid increased flow (24%) but caproic acid did not (Table 10). Qualitatively, the vascular effects of oleic acid and caproic acid in 10% bile were similar to those observed with the two fatty acids in 10 mM taurocholate solution, i.e., oleic acid increased flow but caproic acid did not (Tables 5 and 6). In 33% bile, both fatty acids increased flow, but the hyperemic effects of 20 mM oleic acid (20 - 24%) were greater than those of 20 mM caproic acid (11 - 13%) (Table 8). Thus, the long chain fatty acid produces a greater hyperemia than the short chain fatty acid regardless of the bile concentration. The concentration of the fatty acid is also a factor in the hyperemia because in the presence of bile 40 mM oleic acid produced

twice the hyperemia as did 20 mM oleic acid (Table 8). Thus, the vasodilation produced by fatty acids in the presence of bile is a phenomenon which may not only be dependent on the concentration of bile, but also on the chain length and/or concentration of the fatty acids.

The influence of bile on the hyperemic effects of lipids appears to be limited only to the hydrolytic products of lipid digestion. Triolein (triglyceride composed of oleic acid) did not alter flow whether bile was present or not (Table 11). Also, when triolein was digested it did not increase flow unless bile was added to it (Table 11). Oleic acid did not increase flow unless either bile or taurocholate, a bile salt was present (Tables 5 - 10). These findings suggest that lipids must be digested and their lipolytic products solubilized in bile or bile salts before they produce a hyperemia.

Fara et al. (38) have shown that an intestinal hyperemia can be elicited by the intraduodenal instillation of triglycerides (corn oil). This finding is in conflict with that of the present study. In their study, the latency of the hyperemic response to corn oil (7 - 20 minutes) was longer than that of the hyperemic response to a product of protein digestion, phenylalanine (1 - 5 minutes). Thus, it is possible that the duodenal milieu may have contained bile and pancreatic enzymes and the longer latency period observed for corn oil may be due to the fact that triglycerides must be digested and solubilized before they can produce a hyperemia.

In addition to lipids, bile also alters the vascular effects of the water soluble nutrients, glucose and amino acids. Without bile, glucose increased flow by 5 - 6% and in the presence of bile the hyperemic effect of glucose was significantly enhanced; in 10% bile, glucose increased flow by 10%, and

in 33% bile, glucose increased flow by 22% (Tables 8 and 10). The hyperemic effect of glucose in 10% bile and 33% bile were significantly different (unpaired Student's t-test). Thus, as the concentration of bile is increased from 10 to 33%, there is a greater enhancement of the hyperemic effect of glucose. The 25.2 mM mixture of 16 amino acids did not alter flow whether placed in the lumen alone or in 10% bile. However, in 33% bile the amino acids increased flow by 12%. Thus, the hyperemic effect of luminal glucose and amino acids is dependent on the concentration of bile present in the lumen.

In summary, the vascular effects of various hydrolytic products of food digestion were altered by bile in a concentration dependent manner. At the higher concentration (33%), bile altered the vascular effects of all the hydrolytic products of food digestion (Tables 7 and 8). At the lower concentration (10%), bile altered the vascular effects of only glucose and a long chain fatty acid (Tables 9 and 10). Because bile per se, either 10% or 33%, did not alter local jejunal blood flow (Tables 7 and 9), these effects of bile are not due to a vasoactive property of bile.

The strong possibility that in the intestinal lumen there is an unstirred water layer adjacent to the mucosal cell surface suggests a possible mechanism by which bile allows the water insoluble fatty acid to produce a hyperemia. This unstirred water layer, which has no fixed anatomical dimension, separates the mucosal cell membrane from the well-mixed, bulk contents of the intestinal lumen (109, 112). In this layer, transport of molecules from the bulk intestinal contents to the mucosal cell, or vice versa, occurs only by diffusion (112). Bile, or bile salts, form micelles in which the water insoluble long chain lipolytic products can be effectively

solubilized (61, 62, 93, 109). This allows the lipids to come in contact with the mucosal cell surface by overcoming the resistance to diffusion offered by the unstirred water layer (108). Once in contact with the mucosal cell surface the lipids can 1) be absorbed or 2) interact with specific mucosal endocrine cell receptors or neural receptors. Either of these processes may be involved in the hyperemia (17, 39, 63, 87, 96, 97, 103, 104).

The mechanism by which bile enhances the hyperemic effect of glucose and allows amino acids to produce a hyperemia is not known, but, as was the case for lipids, the unstirred water layer may be involved. The existence of an unstirred water layer causes an underestimation of passive permeability coefficients for passively absorbed substances (110) and an overestimation of the Michaelis constant for actively transported substances (33, 110). Stirring the medium of everted intestinal sacs decreases the Michaelis constant for actively transported substances and increases the passive permeability coefficient for passively absorbed substances by decreasing the thickness of the unstirred water layer (110, 112). This data prompted Dietschy et al. (110) to suggest that "in the presence of a significant unstirred water layer the concentration of a particular molecule at the aqueous-lipid interface is reduced below the concentration of the molecule in the bulk perfusion solution." Bile and bile salts have been observed to produce contractions of the villi (107), which might decrease the thickness of the unstirred water layer by a "stirring" effect of the contracting villi. The end result would be an increase in the concentration of glucose or amino acids at the mucosal cell surface, which in turn, might enhance 1) their absorption or 2) their interaction with specific mucosal

receptors. Again, either of these processes may be involved in the hyperemia.

The hypothesis that bile enhances the hyperemic effects of glucose and allows amino acids to produce a hyperemia by increasing their effective concentration at the mucosal cell surface is speculative. Nonetheless, there are facts which lend credence to the hypothesis. Kvietys et al. (70) have shown that the postprandial intestinal hyperemia requires the presence of a certain amount of digested food in the lumen and is directly related to the concentration of digested food. Sit et al. (95) have shown that addition of bile to digested food enhances the hyperemic effect of digested food. Chou et al. (21) have shown that the hyperemic effect of luminal glucose is progressively enhanced as the concentration of glucose is progressively increased. In the present study, the hyperemic effect of glucose is progressively enhanced as the concentration of bile is increased from 10 to 33% (Tables 8 and 10). Amino acids did not increase flow (Table 2) unless their concentrations were increased ten fold (Table 3) or bile was added to them (Table 10). These findings do suggest that bile may be exerting its influence on the vascular effects of water soluble nutrients and increasing the concentration of these nutrients at the mucosal cell membrane by decreasing the thickness of the unstirred water layer.

As mentioned earlier, exposure of nutrients to the mucosal cell surface can result in 1) absorption of nutrients and/or 2) an interaction of nutrients with specific mucosal receptors. Either of these two events, or both, may be involved in the production of the postprandial intestinal hyperemia and thus bile could alter the vascular effects of nutrients by

altering their absorption or interaction with receptors. In the present study, the possiblity that bile alters the vascular effects of glucose and oleic acid through an enhancement of their absorption was examined.

Glucose (200 mM) with and without 33% bile increased local jejunal blood flow, glucose absorption and oxygen consumption (Tables 12 and 13 and Figure 3). Varro et al. (102) have also shown that there is an increase in jejunal oxygen consumption, blood flow and glucose absorption when 300 mM glucose is placed in the lumen. The increase in oxygen consumption is probably a result of an increase in mucosal cell aerobic metabolism subsequent to 1) the active transport of glucose (27, 49, 72) and 2) the catabolism of the absorbed glucose (31). Glucose absorption and oxygen consumption were increased to the same extent whether glucose was in the lumen alone or with 33% bile (Figure 3). Glucose absorption was also not affected by 10% bile (Table 14). Others have shown that bile or conjugated bile salts do not alter or slightly inhibit glucose absorption occurring in the rat jejunum containing 10 and 20 mM glucose solutions (46, 55, 91). Although glucose absorption was not affected by bile, the hyperemic effect of glucose was enhanced by bile (Figure 3 and Table 14). Thus, it does not appear that bile enhances the hyperemic effect of glucose through an enhancement of glucose absorption.

Oleic acid was absorbed from the jejunal lumen and accumulated by the mucosa whether bile was present or not (Figures 4 and 5). However, in the presence of bile, a greater percentage of oleic acid was absorbed from the lumen and accumulated by the mucosa (Figures 4 and 5). Others have also shown that nonmicellar oleic acid can be absorbed from the upper small intestine, but that the rate of absorption was greater if oleic acid

was in micellar form (61, 69). Thus, these experiments indicate that indeed bile enhances oleic acid absorption. However, the absorption of oleic acid was not always related to its vascular effects. In the absence of bile, oleic acid at pH 7.0 was absorbed from the lumen, but did not increase local jejunal blood flow (Figure 4). At pH 9.5, oleic acid was absorbed from the lumen to a greater extent when bile was present, but the hyperemic effect of oleic acid was the same whether bile was present or not (Figure 5). Thus, although bile enhances oleic acid absorption, the hyperemic effects of oleic acid are not related to its absorption.

Bile could alter the vascular effects of glucose or oleic acid through another mechanism. Bile or bile salts may decrease the resistance to diffusion offered by the unstirred water layer by causing contractions of villi (107) and/or solubilizing lipids (62). These effects of bile or bile salts could increase the chances of interaction between luminal nutrients and receptors which stimulate the release of vasoactive hormones or initiate a neural reflex to produce a local hyperemia. Both neural (21, 87, 103, 105, 114) and/or humoral (38, 63) mechanisms have been implicated in the hyperemia occurring while nutrients are in the lumen.

Finally, it is possible that the mixing of nutrients with bile may have allowed bile, or some constituent of bile, to produce a hyperemia. Although bile per se did not alter jejunal blood flow (Tables 7 - 10), bile does increase blood flow in the ileum (71), the major site of bile salt absorption (31). Furthermore, i.a. infusions of taurocholate, a bile salt, in a constant flow preparation of the jejunum or ileum decreased vascular resistance (96). Therefore it is possible that mixing of nutrients with bile

might enhance the absorption of bile salts and produce a hyperemia via an action of bile salts on local blood vessels.

In conclusion, the hydrolytic products of food digestion are the stimulus for the postprandial intestinal hyperemia. When physiological concentrations of glucose, amino acids, peptides or fatty acids were placed in the jejunal lumen without bile or bile salts, only glucose increased flow. Bile plays an important auxiliary role in the postprandial intestinal hyperemia by altering the vascular effects of the hydrolytic products of food digestion. In general, the effects of bile are concentration dependent; 33% bile alters the vascular effects of all nutrients tested (Tables 7 and 8), while 10% bile only affects glucose and oleic acid (Tables 9 and 10). The duodenal and proximal jejunal concentrations of bile acid (a constituent of bile) can rise to 10 - 17 mg/ml during gall bladder emptying and then decline to 2 - 5 mg/ml for the rest of the digestive period (14). The concentration of bile acids in gall bladder bile is about 48.5 mg/ml (80). Thus, the 33% bile probably represents the peak luminal concentration of bile reached during gall bladder emptying (½ to 1 hour after a meal) while the 10% bile probably represents the bile concentration during the second and following hours of digestion. Thus, during the first half hour after a meal, bile contributes to the postprandial intestinal hyperemia by enhancing the hyperemic effects of glucose and allowing amino acids and the lipolytic products, fatty acids and monoglycerides, to produce a hyperemia. During the remainder of the digestive period, the bile concentration decreases, and it contributes to the hyperemia by altering the vascular effects of hydrolytic products of carbohydrate and long chain lipid digestion only. Since the postprandial intestinal hyperemia persists for up

to 5 - 7 hours after ingestion of a meal (103, 104, 105), it would appear that the major contribution of bile to this hyperemia is through its enhancement of the hyperemic effects of glucose and its permissive role in the hyperemic effects of long chain lipids. The mechanism by which bile enhances the hyperemic effect of glucose and allows oleic acid to produce a hyperemia is not clear, but it is not due to an enhancement of glucose or oleic acid absorption.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Blood flow to the small intestine increases after meals and the hyperemia appears to be confined to that portion of the intestine exposed to chyme. It has been shown that the constituents of chyme responsible for the hyperemia are the hydrolytic products of food digestion and that bile markedly enhances the hyperemic effect of the digested food. The specific hydrolytic products responsible for the hyperemia, however, have not been identified. The major aims of the present study were to identify the hydrolytic products of carbohydrates, proteins, and lipids which are responsible for the hyperemia and to determine whether bile can influence the vascular effects of these products. The venous outflows of two in situ jejunal segments were simultaneously measured while various food chemicals with and without bile were placed into the jejunal lumen. In addition, the effects of bile on the absorption of glucose and oleic acid were studied. The following observations and conclusions were made from these studies.

1. A mixture containing physiological concentrations of glucose (150 mM), 16 amino acids (25.2 mM), and micellar long chain lipids (40 mM oleic acid, 20 mM monoolein, and 10 mM taurocholate) increased local blood flow when placed in the jejunal lumen. Individually, only glucose (150 or 200 mM) increased flow, monoolein (20 mM) and oleic acid (40 mM) required the presence of taurocholate (10 mM) to increase flow, and a 37.5 mM mixture of 3 dipeptides or a 25.2 mM mixture of 16 amino acids

did not alter flow. Thus, in the absence of taurocholate, or bile, only the hydrolytic products of carbohydrates increase intestinal blood flow, while the hydrolytic products of lipids and proteins do not.

2. The amino acids increased flow when the concentration of amino acids was increased from 25.2 to 252 mM. Of the 16 amino acids only glutamic (28 mM) and aspartic (20 mM) acids increased flow. The other 14 amino acids (glycine, valine, leucine, isoleucine, threonine, plenylalanine, tyrosine, tryptophan, cystine, methionine, histidine, lysine, arginine, and proline) did not alter flow. Thus, only two of the 16 amino acids increased flow, and their concentrations had to be raised to 10 times those found in the lumen postprandially.

3. Triglycerides had to be digested and solubilized in bile before they produced a hyperemia. In 33% bile, 40 mM oleic acid produced twice the hyperemia as did 20 mM oleic acid. Oleic acid also produced twice the hyperemia as did an equimolar concentration of caproic acid. In 10% bile, or 10 mM taurocholate, oleic acid increased flow while caproic acid did not. Thus, both concentrations of bile allowed fatty acids to produce a hyperemia and the magnitude of the hyperemia was related to the concentration and chain length of the fatty acid.

4. The hyperemic effects of 200 mM glucose were doubled by 10% bile and tripled by 33% bile. The 25.2 mM amino acids and 37.5 mM dipeptides did not alter flow in the presence of 10% bile, but the amino acids did increase flow in the presence of 33% bile. Thus, both concentrations of bile enhance the vascular effects of glucose, while only the high concentration of bile allowed amino acids to produce a hyperemia.

5. Blood flow, glucose absorption (A-V glucose x blood flow) and oxygen consumption (A-V oxygen x blood flow) of the jejunum increased when 200 mM glucose was in the lumen. Both 10 and 33% bile enhanced the hyperemic effect of glucose, but neither 10 nor 33% bile altered glucose absorption. Jejunal oxygen consumption while glucose was in the lumen was the same whether 33% bile was present or not. Thus, the enhancement of the hyperemic effects of glucose by bile is not due to an effect of bile on glucose absorption.

6. At a pH of 7.0, oleic acid produced a hyperemia only in the presence of 10% bile. Oleic acid-1-^{14}C was absorbed whether or not bile was present, but a greater amount of oleic acid-1-^{14}C was absorbed in the presence of bile. At pH 9.5, oleic acid produced the same hyperemia whether or not bile was present, but bile enhanced the absorption of oleic acid-1-^{14}C. Thus, the hyperemia produced by oleic acid in bile does not appear to be related to oleic acid absorption.

In conclusion, of the major hydrolytic products of carbohdrate, lipid, and protein digestion, only glucose can produce a hyperemia in the absence of bile or taurocholate. The postprandial luminal milieu includes bile and the role of bile in the postprandial intestinal hyperemia is related to its ability to alter the vascular effects of the hydrolytic products of food digestion. The effect of bile appears to be concentration dependent; in 33% bile, all hydrolytic products of food digestion increased flow, while in 10% bile only the hydrolytic products of carbohydrate and long chain lipid digestion increased flow. Thus, it appears that the hydrolytic products of carbohydrates and long chain lipids contribute greatly to the postprandial hyperemia; whereas the hydrolytic products of protein and short chain lipid

digestion contribute little. The ability of bile to enhance the hyperemic affects of glucose and allow oleic acid to produce a hyperemia is not due to its effect on glucose or oleic acid absorption.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Abramson, D. I., and S. M. Fierst. "Peripheral vascular responses in man during digestion." Am. J. Physiol. 133: 686-693, 1941.

2. Adibi, S. A., and D. W. Mercer. "Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentrations after meals." J. Clin. Invest. 52: 1586-1594, 1973.

3. Annegers, J. H. "Some effects of hexoses on the absorption of amino acids." Am. J. Physiol. 210: 701-704, 1966.

4. Altman, P. L., and D. S. Dittmer, editors. Metabolism, Federation of American Societies for Experimental Biology. Bethesda, Md. 1968.

5. Bean, J. W., and M. M. Sidky. "Effects of low O_2 on intestinal blood flow, tonus, and motility." Am. J. Physiol. 189: 541-547, 1957.

6. Biber, B., J. Fara, and O. Lundgren. "Intestinal vascular responses to 5-hydroxtryptamin." Acta. Physio. Scand. 87: 526-534, 1973.

7. Biber, B., J. Fara, and O. Lundgren. "A pharmacological study of intestinal vasodilator mechanisms in the cat." Acta. Physiol. Scand. 90: 673-683, 1974.

8. Biber, B., O. Lundgren, and J. Svanoik. "Studies on the intestinal vasodilation observed after mechanical stimulation of the mucosa of the gut." Acta. Physiol. Scand. 82: 177-190, 1971.

9. Bihler, I. "Sugar transport at the basolateral membrane of the mucosal cell." In Intestinal Permeation proceedings of the fourth workshop conference. Ed. M. Kramer and F. Lauterbach. American Elsevier Pub. Co., New York, 1977.

10. Bihler, I., and P. C. Sawh. "The role of energy metabolism in the interaction between amino acid sugar transport in the small intestine." Can. J. Physiol. Pharmacol. 51: 378-382, 1973.

11. Black, J. W., E. W. Fisher, and A. N. Smith. "The effects of precursors of 5-hydroxytryptamine on gastric secretion in anesthetized dogs." J. Physiology (London) 146: 10-17, 1959.

12. Bloom, S. R. "Hormones of the gastrointestinal tract." Br. Med. Bull. 30: 62-67, 1974.

13. Borgstrom, B. "Digestion and absorption of lipids." In International Review of Physiology Gastrointestinal Physiology II, Volume 12, Ed. Robert K. Crane, University Park Press, Baltimore, Md., 1977.

14. Borgstrom, B., A. Dahlquist, G. Lundh, and J. Sjovall. "Studies of intestinal digestion and absorption in the human. J. Clin. Invest. 36: 1521-1536, 1957.

15. Brandt, J. L., L. Castleman, H. D. Ruskin, J. J. Greenwald, and J. Kelly. "The effect of oral protein and glucose feeding on splanchnic blood flow and oxygen utilization in normal and cirrhotic subjects." J. Clin. Invest. 34: 1017-1025, 1955.

16. Brindley, D. N., and G. Hubscher. "The effect of chain length on the activation and subsequent incorporation of fatty acids into glycerides by the small intestinal mucosa." Biochim, Biophys. Acta. 125: 92-105, 1966.

17. Brodie, T. G., W. C. Cullis, and W. D. Halliburton. "The gaseous metabolism of the small intestine" Part II. "The gaseous exchanges during the absorption of Witte's peptone." J. Physiol. 40: 173-189, 1910.

18. Burns, G. P., and W. G. Schenk. "Effect of digestion and exercise on intestinal blood flow and cardiac output." Arch. Surg. 98: 790-794, 1969.

19. Caspary, W. F. "Mechanism and specificity of intestinal sugar transport." In Intestinal Permeation, proceedings of the fourth workshop conference. Ed. M. Kramer and F. Lauterbach. American Elsevier Pub. Co., New York, 1977.

20. Chen, W. T. "Blood flow in the canine ileum as affected by luminal isosmotic ane hyperosmotic solutions." M.S. Thesis, Michigan State University, East Lansing, Michigan, 1970.

21. Chou, C. C., T. D. Burns, C. P. Hsieh, and J. M. Dabney. "Mechanisms of local vasodilation with hypertonic glucose in the jejunum." Surgery 71: 380-387, 1972.

22. Chou, C. C., C. P. Hsieh, and J. M. Dabney. "Effects of acid and hyperosmolarity of the canine duodenum on local blood flow and motility." Gastroenterology 58: 934, 1970.

23. Chou, C. C., C. P. Hsieh, and J. M. Dabney. "comparison of vascular effects of gastrointestinal hormones on various organs." Am. J. Physiol. 232: H103-H109, 1977.

24. Chou, C. C., and E. C. Texter, Jr. "Comparison of effects of Fe^{++}, Fe^{+++}, and ferritin on intestinal circulation." Am. J. Physiol. 210: 1447-1450, 1966.

25. Chou, C. C., C. P. Hsieh, T. D. Burns, and J. M Dabney. "Effects of lumen pH and osmolarity on duodenal blood flow and motility." Gastoenterology 60: 648, 1971.

26. Chou, C. C., C. P. Hsieh, Y. M. Yu, P. Kvietys, L. C. Yu, R. Pittman, and J. M. Dabney. "Localization of mesenteric hyperemia during digestion in dogs." Am. J. Physiol. 230: 583-589, 1976.

27. Crane, R. K. "Na^{+} dependent transport in the intestine and other animal tissues." Fed. Proc. 1000-1006, 1965.

28. Crane, R. K. "Absorption of sugars." In Handbook of Physiology, Volume III, Section 6, Alimentary Canal, Ed. Code, C. F., Washington D. C., 1968, American Physiological Society, pp 1323-1351.

29. Dabney, J. M., J. B. Scott, and C. C. Chou. "Action of adenosine and ATP on ileal wall tension and blood flow." Physiologist 10: 150, 1967.

30. Dagenais, G. R., A. Oriol, and M. McGregor. "Hemodynamic effects of carbohydrate and protein meals in man: rest and exercise." J. Appl. Physiol. 21: 1157-1162, 1966.

31. Davenport, H. W. Physiology of the Digestive Tract Chicago, Year Book Medical Publishers Incorporated, 1977.

32. Dietshy, J. M. "Effects of bile salts on intermediate metabolism of the intestinal mucosa." Fed. Proc. 34: 370, 1975.

33. Dietshy, J. M., V. L. Sallee, and F. A. Wilson. "Unstirred water layers and absorption across the intestinal mucosa." Gastroenterology 61: 932-934, 1971.

34. Dobbins, W. O. "Morphological aspects of lipid absorption." Am. J. Clin. Nutr. 22: 257-265, 1969.

35. Esposito, G., A. Faelli, and V. Capraro. "A critical evaluation of the existence of an outward sugar pump in the basolateral membrane of the enterocyte." In Intestinal Permeation, proceedings of the fourth workshop conference. Ed. M. Kramer and F. Lauterbach. American Elsevier Pub. Co., New York, 1977.

36. Essex, H. E., J. F. Herrick, E. J. Baldes, and F. C. Mann. "Blood flow in the circumflex branch of the left coronary artery of the intact dog." Am. J. Physiol. 117: 271-279, 1936.

37. Fara, J. W., and K. S. Madden. "Effect of secretin and cholecystokinin on small intestinal blood flow distribution." Am. J. Physiol. 229: 1365-1370, 1975.

38. Fara, J. W., E. H. Rubinstein, and R. R. Sonnenshein. "Intestinal hormones in mesenteric vasodilation after intraduodenal agents." A. J. Physiol. 223: 1058-1067, 1972.

39. Fasth, S., S. Filipsson, L. Hulten, and J. Martinson. "The effect of the gastrointestinal hormones on small intestinal motility and blood flow." Experientia 29: 982, 1973.

40. Fordtran, J. S., and T. W. Locklear. "Ionic constituents and osmolality of gastric and small-intestinal fluids after eating." Am. J. Dig. Dis. 11: 503-521, 1966.

41. Fronek, K., and A. Fronek. "Combined effect of exercise and digestion on hemodynamics in conscious dogs." Am. J. Physiol. 218: 555-559, 1970.

42. Fronek, K., and L. H. Stahlgren. "Systemic and regional hemodynamic changes during food intake and digestion in non-anesthetized dogs." Circulation Res. 23: 687-692, 1968.

43. Gallavan, R. H., P. R. Kvietys, S. P. Sit, and C. C. Chou. "Distribution of blood flow during digestion in conscious dogs." Physiologist 20: 31, 1977.

44. Gladstone, S. A. "Cardiac output and related functions under basal and postprandial conditions." Arch. Internal Med. 55: 533-546, 1935.

45. Go, V. L. W., A. F. Hofmann, and W. H. J. Summerskill. "Pancreozymin bioassay in man based on pancreatic enzyme secretion: potency of specific amino acids and other digestive products." J. Clin. Invest. 49: 1558-1564, 1970.

46. Gracy, M., V. Burke, and A. Oshin. "Influence of bile salts on intestinal sugar transport in vivo." Scand. J. Gastroent. 6: 273-276, 1971.

47. Grafe, W. R., and K. G. Swan. "Hemodynamics of the experimental dumping syndrome." Surgery 66: 734-740, 1969.

48. Gray, G. M., and H. L. Cooper. "Protein digestion and absorption." Gastroenterology 61: 535-544, 1971.

49. Gray, S. M "Carbohydrate digestion and absorption. Role of the small intestine." N. Engl. J. Med. 292: 1225-1230, 1975.

50. Grollman, A. "Physiological variations in the cardiac output of man. III. The effect of the ingestion of food on the cardiac output, pulse rate, blood pressure and oxygen consumption of man." Am. J. Physiol. 89: 366-370, 1929.

51. Grossman, M. I. "Gastrointestinal hormones: spectrum of actions and structure activity relations." In Endocrinology of the Gut, Ed. W. Y. Chey and R. P. Brooks, Charles B. Slack, Inc., Thorofare, N. J., 1974.

52. Haddy, F. J., C. C. Chou, J. B. Scott, and J. M. Dabney. "Intestinal vascular responses to naturally occurring vasoactive substances." Gastroenterology 52: 444-451, 1967.

53. Haddy, F. J., and J. B. Scott. "Metabolically linked vasoactive chemicals in local regulation of blood flow." Physiol. Rev. 48: 688-707, 1968.

54. Haddy, F. J., and J. B. Scott. "Metabolic factors in peripheral circulatory regulation." Fed. Proc. 34: 2006-2011, 1975.

55. Harries, J. T., and G. E. Sladen. "The effects of different bile salts on the absorption of fluid, electrolytes, and monosaccerides in the small intestine of the rat in vivo." Gut 13: 596-603, 1972.

56. Herrick, J. F., H. E. Essex, R. C. Mann, and E. J. Baldes. "The effect of digestion on blood flow in certain blood vessels of the dog." Am. J. Physiol. 108: 621-628, 1934.

57. Hilton, S. M., and M. Jones. "The role of plasma kinin in functional vasodilation in the pancreas." J. Physiol. 195: 521-533, 1968.

58. Hoffman, A. F. "Functions of bile in the alimentary canal." In Handbook of Physiology, Volume V., Section 6, Alimentary Canal, Ed. Code, C. F., Washington D. C., 1968, American Physiological Society, pp. 1277-1307.

59. Hoffman, A. F., and B. Borgstrom. "The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absortion." J. Clin. Invest. 43: 247-257, 1964.

60. Hoffman, A. F., and D. M. Small. "Detergent properties of bile salts: correlation with physiological function." Ann. Rev. Med. 18: 333-376, 1967.

61. Hoffman, N. E., and A. F. Hoffman. "Absorption rates of micellar vs. non-micellar oleic acid: jejunal perfusion studies in man." Gastroenterology 62: 763, 1972.

62. Holt, P. R. "The roles of bile acids during the process of normal fat and cholesterol absorption." Arch. Intern. Med. 130: 574, 1972.

63. Hsiesh, C. P., and C. C. Chou. "Role of humoral substances in increasing the duodenal blood flow and motility when the acid of foodstuff is in the lumen." Fed. Proc. 31: 391, 1972.

64. Hsieh, C. P., J. M. Dabney, W. T. Chen, and C. C. Chou. "Effect of lumen acidity and osmolarity on duodenal bloodflow and motility." Physiologist 13: 227, 1970.

65. Johnson, L. R. "Gastrointestinal hormones." In MTP International Review of Science, Physiology Series I, Vol. 4, Ed. A. C. Guyton, University Pack Press, Baltimore, Md., 1974.

66. Johnston, J. M. "Mechanism of fat absorption." In Handbook of Physiology Volume III., Section 6, Alimentary Canal, Ed. C. F. Code, Washington D. C. 1968, American Physiological Society, pp. 1353-1375.

67. Kellum, J. M., and B. M. Jaffe. "Validation and application of a radio immunoassay for serotonin." Gastroenterology 70: 516-522, 1976.

68. Kimmich, G. A., and J. Randles. "A Na^+-independent transport system for sugars in intestinal epithelial cells: Specificity kinetics, and interaction with inhibitors." In Intestinal Permeation, proceeding of the fourth workshop conference. Ed. M. Kramer and R. Lauterback. American Elsevier Pub. Co., New York, 1977.

69. Knoebel, L. K. "Intestinal absorption in vivo of micellar and nonmicellar lipids." Am. J. Physiol. 223: 255-261, 1972.

70. Kvietys, P., R. Pittman, and C. C. Chou. "Contribution of luminal concentration of nutrients and osmolality to postprandial intestinal hyperemia in dogs." Proc. Soc. Exp. Biol. Med. 152: 659-663, 1976.

71. Kvietys, P., S. P. Sit, and C. C. Chou. "The role of amino acids, fatty acids, and bile in the postprandial intestinal hyperemia in dogs." Fed. Proc. 36: 623, 1977.

72. Levitan, R., and D. E. Wilson. "Absorption of water soluble substances." In International Review of Physiology, Gastrointestinal Physiology, Volume 4, Ed. E. D. Jacobson and L. L. Shanbour, University Park Press, Baltimore, Md., 1977.

73. MacDonald, J. M., M. M. Webster, Jr., C. H. Tennyson, and Drapanas. "Serotonin and bradykinin in the dumping syndrome." The Am. J. of Surg. 117: 204-211, 1969.

74. Mailman, D., W. Pawlik, A. P. Shepherd, L. L. Tague, and E. D. Jacobson. "Cyclic nucleotide metabolism and vasodilation in canine mesenteric artery." Am. J. Physiol. 232: H191-H196, 1977.

75. Makhlouf, G. M. "The neuroendoerine design of the gut." Gastroenterology 67: 159-184, 1974.

76. Mathews, D. M. "Memorial Lecture: Protein absorption - then and now." Gastroenterology 73: 1267-1279, 1977.

77. Matthews, D. M., and S. A. Adibi. "Peptide Absorption." Gastroenterology 71: 151-161, 1976.

78. Mathews, D. M., and G. Wiseman. "Transamination by the small intestine of the rat." J. Physiol. (London) 120: 55P, 1953.

79. Mohamed, M. S., and J. W. Bean. "Local and general alterations of blood CO_2 and influence of intestinal motility in regulation of intestinal blood flow." Am. J. Physiol. 167: 413-425, 1951.

80. Nakayama, F., and C. G. Johnston. "Solubility of human gallstones in primate gall bladder." Proc. Soc. Exp. Biol. Med. 104: 73-75, 1960.

81. Neame, K. D., and G. Wiseman. "The transamination of glutamic and aspartic acids during absorption by the small intestine of the dog in vivo." J. Physiol. 135: 442-450, 1957.

82. Nixon, S. E., and G. E. Mawer. "The digestion and absorption of protein in man, 1. The site of absorption." Br. J. Nutr. 24: 227-240, 1970.

83. Nixon, S. E., and G. E. Mawer. "The digestion and absorption of protein in man. 2. The form in which digested protein is absorbed." Br. J. Nutr. 24: 241-258, 1970.

84. Norryd, C., H. Dencher, A. Lunderquist, T. Olin, and U. Tylen. "Superior mesenteric blood flow during digestion in man." Acta. Chir. Scand. 141: 197-202, 1975.

85. Olmstead, W. W., E. S. Nassett, and M. I. Kelley, Jr. "Amino acids in postprandial gut contents of man." J. Nutr. 90: 291-294, 1966.

86. Paine, R. M., and N. W. Shock. "The variability of cardiac output estimations made with the high freqency undamped balistocardiograph." Circulation 1: 1026-1031, 1951.

87. Post, J. A., C. C. Chou, P. Kvietys, S. P. Sit, R. Pittman, and J. M. Dabney. "Possible mechanisms of postprandial intestinal hyperemia." Fed. Proc. 34: 459, 1975.

88. Renninger, E. J., and S. Nutik. "Determination of cardiac output following a meal in unanesthetized dogs." Fed. Proc. 19: 118, 1960.

89. Renninger, E. J., and L. A. Sapirstein. "Effect of digestion on distribution of blood flow in the rat." Science 126: 1176, 1957.

90. Robinson, J. W. L., and F. Alvarado. "Comparative aspects of the interactions between sugar and amino acid transport system." In Intestinal Permeation, proceeding of the fourth workshop conference. Ed. M. Kramer and F. Lauterbach. American Elsevier Pub. Co., New York, 1977.

91. Saunders, D. R. "Regional differences in the effect of bile salts on absorption by rat small intestine in vivo." J. Physiol. 250: 373-383, 1975.

92. Schehadek, Z., W. E. Price, and E. D. Jacobson. "Effects of vasoactive agents on intestinal blood flow and motility in the dog." Am. J. Physiol. 216 386-392, 1969.

93. Simmonds, W. J. "Effect of bile salts on the rate of fat absorption." Am. J. Clin. Nutr. 22: 266-272, 1969.

94. Simmonds, W. J. "Absorption of lipids." In International Review of Physiology, Gastrointestinal Physiology, Volume 4, Ed. E. D. Jacobson and L. L. Shanbour, University Park Press, Baltimore, 1974.

95. Sit, S. P., P. Kvietys, and C. C. Chou. "Identification of constituents of chyme responsible for postprandial intestinal hyperemia." Physiologist 19: 369, 1976.

96. Sit, S. P., P. Kvietys, R. Gallavan, C. C. Chou, and D. Collings. "Vascular effects of local i.a. infusion of micellar fatty acids and taurocholate (TCA) in the canine small intestine." Physiologist 20: 28, 1977.

97. Sit, S. P., P. Kvietys, R. Galloran, and C. C. Chou. "Postprandial intestinal hyperemia and oxygen consumption in dogs." Fed. Proc. 37: 653, 1978.

98. Spector, W. S., editor. Handbook of Biological Data, W. B. Saunders Company, Philidelphia, p. 199, 1956.

99. Spencer, R. P. "Intestinal absorption of amino acids." Current Concepts Am. J. Clin. Nutr. 292-299, 1969.

100. Texter, E. C., C. C. Chou, S. L. Merrill, H. C. Laureta, and E. D. Frohlich. "Direct effects of vasoactive agents on segmental resistance of the mesenteric and portal circulation." J. Lab. Clin. Med. 64: 624-633, 1964.

101. VanHeerden, P. O., H. M. Wagner, Jr., and S. Kaihora. "Intestinal blood flow during perfusion of the jejunum with hypertonic glucose in dogs." Am. J. Physiol. 215: 30-33, 1968.

102. Varro, V., L. Csernay, F. Szarvas, and G. Blaho. "Effect of glucose and glycine solution on the circulation of the isolated jejunal loop in the dog." Am. J. Digest. Diseases 12: 60-64, 1967.

103. Vatner, S. F., D. Franklin, and R. L. VanCitters. "Mesenteric vasoactivity associated with eating and digestion in the conscious dog. Am. J. Physiol. 219: 170-174, 1970.

104. Vatner, S. F., D. Franklin, and R. L. VanCitters. "Coronary and visceral vasoactivity associated with eating and digestion in conscious dogs." Am. J. Physiol. 219: 1380-1385, 1970.

105. Vatner, S. F., T. A. Patrick, C. B. Higgins, and D. Franklin. "Regional circulatory adjustments to eating and digestion in conscious unrestrained primates." J. Appl. Physiol. 36: 525-529, 1974.

106. Weisbrodt, N. W. "Bile production, secretion, and storage." In Gastrointestinal Physiology, Ed. L. R. Johnson, C. U. Mosby Company, St. Louis, 1977.

107. Wells, H. S., and R. G. Johnson. "The intestinal villi and their circulation in relation to absorption and secretion of fluid." Am. J. Physiol. 109: 387-402, 1934.

108. Westergaard, H., and J. M. Dietschy. "The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell." J. Clin. Invest. 58: 97-108, 1976.

109. Wilson, F. A., V. L. Sallee, and J. M Dietshy. "Unstirred water layers in intestine: rate determinant of fatty acid absorption from micellar solution." Science 174: 1031-1033, 1971.

110. Wilson, F. A. and J. M. Dietschy. "The intestinal unstirred layer: its surface area and effect on active transport kinetics." Biochimica et Biophysic Acta. 363: 112-126, 1974.

111. Windmueller, H. G., and A. E. Spaeth. "Vascular perfusion of rat small intestine: metabolic studies with isolated and in situ preparation." Fed. Proc. 36: 177-181, 1977.

112. Winne, D. "The influence of unstirred layers on intestinal absorption." In Intestinal Permeation, proceedings of the fourth workshop conference. Ed. M. Kramer and F. Lauterbach. American Elsevier Pub. Co., New York, 1977.