THE EFFECT OF BILE SALT ON THE MOVEMENT OF PALMITIC ACID ACROSS THE GUT OF *ASCARIS LUMBRICOIDES SUUM*

By

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THE EFFECT OF BILE SALT ON THE MOVEMENT OF PALMITIC ACID ACROSS THE GUT OF ASCARIS LUMBRICOIDES SUUM

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CHAPTER I

INTRODUCTION

Most of the literature in the field of digestion and absorption deals with vertebrate organisms. Hence, the introduction for this study reviews information relating mainly to vertebrate processes. The writer also has restricted his discussion to information relating to the digestion and absorption of lipids.

Bile Salts

Bile salts have two important actions in the intestinal tract. First they have a detergent action on the particles in the food, which decreases the surface tension of the particles and allows the agitation in the intestinal tract to break the fat globules into minute sizes. Detergents are substances that possess both fat and water attracting parts. Taurocholate, Fig. 1, a widely distributed bile salt will serve as an illustration.

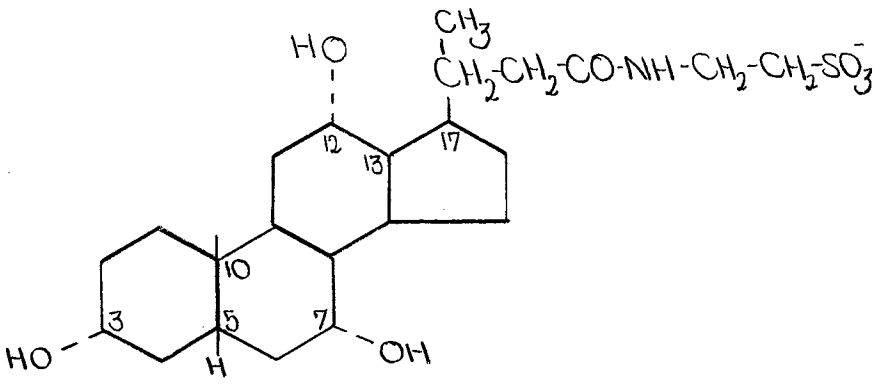

Fig. 1. Taurocholate

The fat soluble portion comprises the steroid ring system and most of the C_{17} side chain. The water-attracting portion consists of the hydroxyl groups at C_3, C_7 and C_{12}, and the SO_3^- group derived from taurine at the end of the side chain. In the presence of these detergents the churning effect of peristalsis results in a progressively finer and finer state of distribution of the dietary lipid in the continuous aqueous phase, facilitating lipolysis. Second and even more important than the emulsifying function, bile salts help in the absorption of fatty acids and monoglycerides from the intestinal tract. This is called their hydrotropic function. It is believed that the negatively charged bile salt ions become physically absorbed to the fatty acids, and the electrical charges of these ions presumably then increase the solubility of these fatty acids, thereby allowing ready passage through the intestinal mucosa (Guyton, 1966). This latter point is substantiated in the work done by Read (1950), who reported that when bile is totally excluded from the intestinal tract as a result of severe liver dysfunction, extrahepatic bilary obstruction or biliary fistula, lipid absorption was markedly impeded, but other lipid-soluble substances were also poorly absorbed.

In man, bile continually elaborated by the polygonal cells of the liver and passes along the bile canaliculi and thence through the hepatic and cystic ducts to the gallbladder. Here it is stored and concentrated and enters the intestine through the common duct when food is present. Emptying of the gallbladder occurs only under the influence of partially digested food in the intestine. In part, this seems to be under neural control, but gallbladder contraction and emptying

may be observed after complete denervation of this organ and introduction of partially hydrolyzed lipid into the duodenum. Acidic extracts of the duodenal mucosa contain a material called cholecystokinin, a hormone released by the small intestine, which stimulates contraction of the gallbladder with release of its contents into the duodenum. The primary constituents found in human bile are listed in Table I.

TABLE I

THE COMPOSITION OF HUMAN BILE
(PARTS PER 1,000)

Constituent	Fistula Bile	Bladder Bile
Water	976.0	860.0
Solids	24.0	140.0
Bile Acids	5.7	53.7
Mucin & Pigments	8.0	41.4
Total Lipids	2.9	18.8
Inorganic Matter	7.4	8.5

The capacity of the gallbladder is 50 to 60 ml. in adults. The gallbladder not only is a storage sac but also concentrates bile by absorption of water, bile salts and electrolytes and secretes mucoproteins (White et.al., 1959). In animals which possess no gallbladder, the bile passes directly from the hepatic duct through the common bile duct to the duodenum (Oser, 1965). The bile acids, synthesized in the liver, are the chief, if not the only, contribution of bile to

digestion; these acids are present in bile as bile salts. Table II shows the distribution and gives the common names of a few principal bile acids.

TABLE II

PRINCIPAL BILE ACIDS EXCRETED IN BILE

Species	Common Name	%
Human	Cholic Acid	41
	Chenodeoxycholic Acid	37
	Deoxycholic Acid	22
Rat &/or Mouse	Cholic Acid	80
	Chenodeoxycholic Acid	18
Rabbit	Deoxycholic Acid	90
	Cholic Acid	10
Pig	Hyocholic Acid	
	Hyodeoxycholic Acid	
	Chenodeoxycholic Acid	

Except at very great dilutions, bile salt molecules clump together as micelles. In defining michelle it is known that solutions of certain substances, which in the same molecule contain a dissymetric nonpolar and polar regions, may have peculiar properties. Such a compound is called amphipathic and show a tendency to accumulate at interfaces. When their concentration increases over a certain critical level they associate into small molecular aggregates called association

colloids or micelles. Micelles are probably spherical and have an interior of fluid hydrocarbon with polar groups orientated at the surface. Micellar solutions have long been known to be able to solublize nonpolar substances, such as hydrocarbons in their liquid interior (Hartley, 1955). The expanded bile acid-unsaturated monoglyceride micelles can solubilize more nonpolar compound that the bile salt micelle alone, and the soulbilization increases with an increasing amount of monoglyceride in the micelle. Monoglycerides and fatty acids are responsible for the lowering of the interfacial tension and emulsion stability, while the bile salts provide the negative charge on the particles.

Bile acids are synthesized from cholesterol, Fig. 2, exclusively by the hepatic mitochondria and represent one of the major end products of cholesterol metabolism.

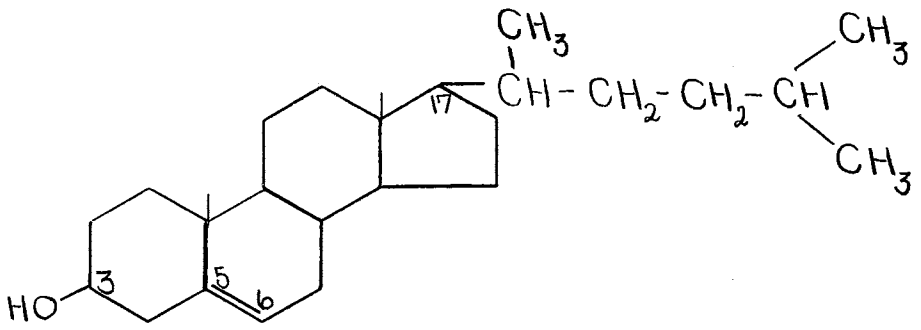

Figure 2. Cholesterol

The metabolism of cholesterol to bile acids proceeds in a definite sequence of several reactions. The order of the initial steps is uncertain, but it is believed that the first reaction is the stero-specific introduction of a hydroxyl group at position 7 and perhaps

hydroxylation of C_{26}. This is followed by stereospecific saturation of the Δ^5 double bond and the inversion of the 3-β-hydroxyl which involves ketone formation and reduction. A α-hydroxyl group is then introduced at the 12 position. Only after hydroxylation of the ring has reached the 3α-, 7α-hydroxyl stage can oxidation of the terminal carbons of the isooctyl side chain go to completion. After the introduction of the hydroxyl at C_{26}, partial ring hydroxylation and saturation, the C_{26}, is further oxidized to a carboxyl group. This is followed by β- oxidation and cleavage of a 3- carbon acid or its CoA derivative (Oser, 1965). Cholesterol is probably the chief or only sterol concerned in most cases. Conversion to bile salts is the only major method by which cholesterol is degraded in vivo. Loss by this route and the excretion of cholesterol itself in the bile thus comprises in many species, including man, almost the only means by which the body cholesterol can be diminished.

Spencer (1964) has shown that bile salts are absorbed most rapidly in the lower regions of the ileum. Fats on the other hand, are effectively absorbed in the upper regions of the small intestine. If if is true that bile salt and fat absorption occur at separate sites, then the concepts which have been developed pertaining to micelle formation and the possible intracellular metabolic effects of bile salts require that there be a blockage of bile salt transport into the circulation. This blockage occurs at the site of fat absorption and the bile salts are secreted or diffuse back into the lumen at that point. Lack & Weiner (1963) have reviewed their work and discussed the lumen of the proximal small gut where fat absorption is the greatest.

Bile salts are then reabsorbed in the ileum after fat absorption is complete. From the ileum they are recirculated, via the portal blood, to the liver and then the bile where they re-enter the duodenum. Borgstrom, Lundh & Hofmann (1963) found that in man net bile salt absorption is negligible in the proximal small gut but significant distally. This point is in agreement with Baker & Searle (1960) in the rat and Lack & Weiner (1963) in rats and guines pigs. Playoust and Isselbacher (1963) have confirmed the absence in the jejunum of active transport mechanisms for bile salt absorption, but found the mechanism present in rat and hamster ileum. They have shown also that no hydrolysis or formation of the peptide conjugates occurs in the intestinal mucoso.

Hofmann and Borgstrom (1962) estimated the bile salt range found in the intestinal content during digestion in man to be 5-10 mEq. liter of content. This value is supported by White, Handler & Smith (1964) who reported the daily secretion of bile salts to be 5-15 gr./day.

The Vertebrate Gut

The vertebrate gut is made up of two major parts: a proximal portion called the small intestine, and the distal part, the large intestine. The small intestine extends from the pylorus to the cecum, and the large intestine extending from its junction with the small intestine to the anus. Most absorption of nutrient material occurs in the small intestine while the absorption of water occurs in the large intestine. The small intestine is divided into three regions, the duodenum, jejunum and ileum. The duodenum is the first or proximal

portion and extends from the pylorus to the jejunum. The jejunum accounts for the mid-portion of the intestine. The ileum is the distal portion of the small intestine and extends from the jejunum to the cecum. The absorbing surface of the gut is greatly increased by intensive infolding. Further, increases in the surface area is due to the finger-like projections called villi which are located over most of the surface of the small intestine from approximately the point at which the common bile duct empties into the duodenum down to the ileocecal valve. They project about 1 millimeter from the surface of the mucosa. Villi lie so close to each other in the upper small intestine that they actually touch in most areas, but their distribution is less profuse in the distal small intestine. The entire surface of the villi are covered by specialized epithelium. The epithelial cell membrane that is exposed to the lumen of the intestine is formed into numerous projections called microvilli and is collectively known as the brush border of the intestinal mucosa. Obviously these anatomical specializations are designed to increase the surface area of the gut which is exposed to the intestinal materials.

Dietary Lipids

By far the most common fats of the diet are the glycerides, with the triglyceride being the most abundant. Each triglyceride is composed of a glycerol "backbone", with three fatty acids. The fatty acids are bound to the glycerol by an ester bond. In addition to the fats, the usual diet also contains small quantities of phospholipids, cholesterol, and the cholesterol esters. The phospholipids and

cholesterol esters contain fatty acid and, therefore, can be considered as fats themselves. Cholesterol, on the other hand, is a sterol compound containing no fatty acid, but it does exhibit some of the physical and chemical characteristics of fats; it is derived from fats, and it is metabolised similarly to fats. Therefore, cholesterol is considered from a dietary point of view to be a fat. Triglycerides represent a useful form of lipid which is hydrophobic, chemically unreactive, of relatively low density, and composed of a great many hydrogen atoms per molecule. These characteristics make the triglycerides a most convenient storage form of energy and of long-chain fatty acids for the mobile mammalian species. Our diets include triglycerides stored in tissues of animal food sources. Yet triglycerides, the preferred storage and shipping form of fat, seem to be hard to move across membranes. Efficient movement of fats require their breakdown into a more reactive and relatively more polar form. (Florkin & Stotz, 1963).

Fat Digestion

The first step in fat digestion is to disperse the fat globules into small sizes so that the water-soluble digestive enzymes can act on the particle surface. This process is called emulsification, and it is achieved under the influence of bile. Division of the food triglyceride into a coarse emulsion occurs principally in the stomach by its mechanical squirting and churning movements. In this process the fats are mixed with phospholipids and other chyme components. When the fat emulsion is ejected into the duodenum, it mixes with bile and

pancreatic juice. In the small intestine hydrolysis of the triglycerides is mediated by pancreatic lipase. This is by far the most important enzyme for the digestion of fats. The epithelial cells of the small intestine, however, contain a small quantity of lipase known as enteric lipase. Both enzymes act alike to cause hydrolysis of fat (Guyton, 1964). Some research workers believe that most dietary triglycerides are finally broken down into fatty acids and glycerol. The majority of the investigators believe, however, that more than half of the triglycerides are digested only to mono and diglyceride stages. Recent quantitative studies (Reiser & Fu, 1966; Feldman & Borgstrom, 1966) have reconfirmed the predominance of monglycerides and free fatty acids, and the negligible quantitative contribution of intact triglycerides in the mixture of products taken up by the intestine. In considering the fate of a single triglyceride droplet attacked by pancreatic lipase at its oil-water interface, it seems reasonable that a whole family of smaller droplets of triglycerides must result as the fatty acids and 2- monoglycerides are liberated and are complexed with conjugated bile salts to form micelles. It is in this form that the fats are brought in contact with the mucosal cell.

Absorption of Fatty Acids and Monoglycerides

Fats are believed to be absorbed through the intestinal membrane principally in the form of fatty acids and monoglycerides, though a few diglycerides and triglycerides are also absorbed. It is not clear whether the micelles with their bile salt and lipid moieties enter the mucosal cell as such or whether the fatty acids and monoglycerides,

are released from their micellar state at the outer membrane of the mucosal cell (the microvilli) and enter in some other physiochemical form. Generally though it is believed that fatty acids are absorbed in the following manner: The fatty acid molecule, being highly lipid-soluble, becomes dissolved in the membrane of the brush border of the epithelial cell and diffuses to the interior of the cell. There it comes in contacy with the endoplasmic reticulum, which uses fatty acid molecules to resynthesize triglycerides similar to those from which the fatty acid had been derived by digestion in the intestine. The absorption of monoglycerides, and perhaps small quantities of diglycerides, occurs in very much the same way as the absorption of fatty acids. Chemical reactions for the synthesis of triglycerides are different, however, since the glycerol portion of the triglyceride is already present.

Speaking in general terms, there are two basic mechanisms involved in the absorption process, one is active transport and the other is diffusion. Active transport is believed to occur most often in the following manner. First the substance to be transported combines with a carrier at one surface of the cell membrane. This combination then diffuses through the membrane to the opposite side where the substance is released from the carrier. The carrier then returns to the first surface to pick up still more substance. When the substance combines initially with the carrier or when it is released from the carrier, energy is expended and the movement of material can occur against an electrochemical gradient. Unfortunately, the precise mechanism of even a single carrier system for active transport has

not been worked out. The term "diffusion" means simply movement of substances through the membrane as a result of molecular movement along, rather than against, an electrochemical gradient. In some instances material is moved by a process called "facilitated diffusion". This means that a carrier mediated process allows the substance to move through the membrane more rapidly that it would by simple diffusion. Facilitated diffusion differs from active transport in that it is incapable of moving substances against an electrochemical gradient. On the other hand, it is similar to active transport in that it involves specific selectivity of material to be passed through the membrane. After entry into the cell both fatty acids and monoglycerides are esterified to triglycerides and this occurs by two major metabolic routes. One pathway involves the acylation of fatty acids to Acyl-CoA triolesters which then react with L-α- Glycerophosphate to yield phosphatidic acid derivatives. The other route involves the direct interaction of monoglycerides with fatty Acyl-CoA molecules to yield diglycerides and triglycerides. There is an increase in water solubility when a fatty acid is transformed to its CoA thiolester derivative. When the activated fatty acid and monoglyceride condense to form di- and tri- glycerides the reverse happens, that is, the water solubility decreases sharply. At some point during the passage of fat through the cell this occurs, since ingested fatty acids are converted to triglycerides before they enter the lymph. Figure 3 illustrates the major chemical steps involved in the synthesis of triglycerides by the endoplasmic reticulum.

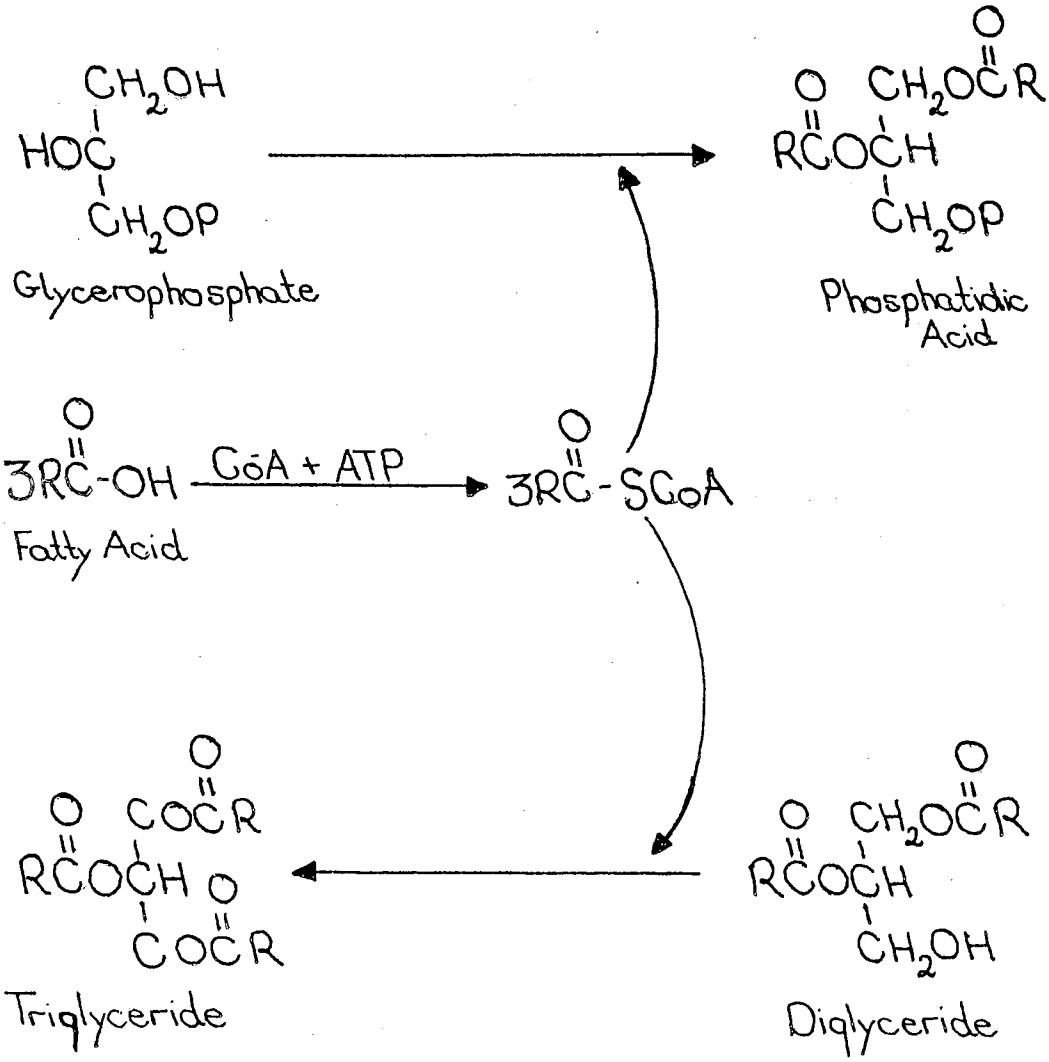

Figure 3. Chemical Steps in the Resynthesis of Triglycerides by the Endoplasmic Reticulum.

Intracellular triglyceride synthesis from fatty acids and partial glycerides in the intestinal mucosa may be facilitated by conjugated bile salts as suggested by Dawson and Isselbacher (1960). Upon incubating slices of rat intestive in the presence of taurocholate, they found that incorporation of C^{14} palmitate into tissue triglycerides was greater than when such slices were incubated in the absence of taurocholate.

After absorption from the intestinal tract, fatty acid molecules with chain lengths of ten carbon atoms or less are known to be transported unesterified in the portal rather than the lymphatic system. The preferential transport of these lipids via the portal blood stream as free fatty acids may be related to their poor incorporation into mucosal triglycerides (Dawson & Isselbacher, 1960). In contrast to medium-chain fatty acids, longchain fatty acids are converted to triglycerides and secreted in the lumph in the form of lipoproteins. These are very low density lipoproteins known as chylomicrons, which contain small amounts of phospholipid and cholesterol in addition to the protein moiety and triglycerides (Brown, 1962). Chylomicrons (0.5 microns in diameter) are coated with a layer of protein, which makes them hydrophilic rather than hydrophobic and allows a reasonable degree of suspension stability in the extracellular fluids. From beneath the epithelial cells the chylomicrons wend their way into the central lactelas of the villi and from here are pumped by the lumphatic pump upward through the thoracic duct to be emptied into the great veins of the neck (juncture of the jugular and subclavian veins) (Guyton, 1964). Between 80 and 90 percent of all fat absorbed from

the gut is absorbed in this manner and transported to the blood by way of the thoracic lymph in the form of chylomicrons. The cause of this difference between short and long chain fatty acid absorption is presumably that the shorter chain fatty acids are more diffusible than the longer ones. The short chain fatty acids are also more water-soluble, which allows direct diffusion of these fatty acids from the epithelial cells into the capillary blood of the villus. Figure 4 is a sketch showing in simplified form the steps in the digestion and absorption of lipids already discussed. In evidence in the drawing is lipolysis, micellar formation, fatty acid and monoglyceride absorption and the recirulation of conjugated bile salts.

Chylomicron lipids are removed from the blood in two ways. Fat is removed by hydrolysis of the chylomicrons triglycerides into glycerol and fatty acids under the influence of an enzyme in the blood called lipoprotein lipase. Glycerol, which is metabolized in much the same way as glucose diffuses into the blood, while the fatty acids complex with albumin and are transported to the various cells of the body. Once the fatty acids are taken into the body cells they may be oxidized for energy, or resynthesized into triglycerides. Triglycerides are then stored in adipose tissue to be used later for energy. Chylomicrons may be removed from the blood by transport through the capillary wall and directly into the cells. In the cells the chylomicron is hydrolyzed and the fatty acids, etc., may be used either for energy or forming new structural compounds (Guyton, 1964).

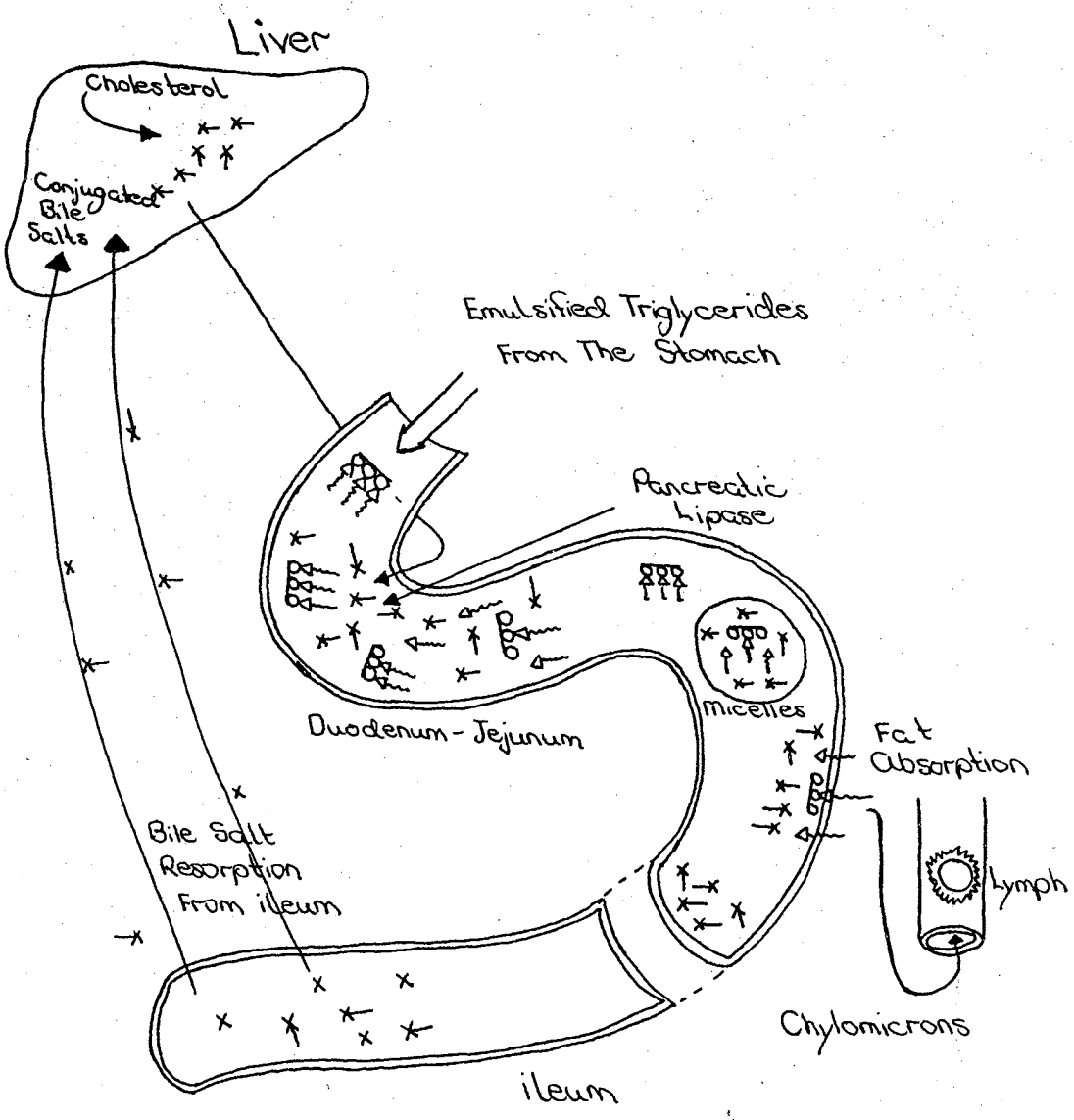

Figure 4. A Scheme of Intraluminal Micelle Formation, Fat and Bile Salt Absorption: x-- Conjugated Bile Salt; x, Unconjugated Bile Salt; , Free Fatty Acid; 000, Free Glycerol

Ascaris

Ascaris lumbricoides suum is a parasitic nematode which inhabits the anterior section of the small intestine of the pig. A physiological variety, Ascaris lumbricoides is found in man. Neither hemoglobin nor its degradation products are found in the intestinal contents of Ascaris. This has led investigators to believe that Ascaris seldom feeds on host tissue. Thus it is highly probable that the parasite feeds on digested material from the lumen of the host's intestine (Rogers, 1940).

This nematode feeds by drawing semi-digested food, bacteria and other intestinal contents into the alimentary system by means of a powerful sucking pharynx. The food is then passed along the pharynx, where secretions from the pharyngeal glands mix with it, to the intestine where digestion and absorption takes place.

The intestine is divided into three regions, the anterior region, the midregion and the posterior (pre-rectal) region. These regions differ from each other in the shape of the lumen, the height and the contents of the cells, and possibly in their function. The wall of the intestine consists of a single layer of cells and there is usually a basement membrane covering the external surface of these cells. The internal surface of the cells is covered with microvilli (Kessel et.al., 1961). The intestinal cells are believed to be both secretory and absorptive in function but there is some evidence that the anterior region is mainly secretory and the mid- and posterior regions mainly absorptive (Carpenter, 1952). The contents of the intestine move about as a result of the locomotory activities of the nematode, and the

ingestion of more food.

Little work has been done on lipid metabolism in nematodes although it is known that nematodes must have an active metabolic system. This was suggested by the quantity of lipid material deposited in the oocytes. Fairbairn and Passey (1957) have shown that there is a definite catabolism of lipid by the developing embryo. Available evidence, however, points to a very low level of fatty acid synthesis in the muscle and ovary-oviduct tissue of Ascaris (Beames, et.al., 1967). So, the question is-how dothe worms obtain the fats that are present in the eggs?

Fats are digested by lipases, which hydrolyse the esters of higher fatty acids, and by esterases, which hydrolyse shorter chain fatty acids. Esterases and lipases are present in the intestine of Ascaris, the esterases being the most active enzyme (Rogers, 1941). There are also lipases and esterases present in the intestinal content that have been secreted by the host which would aid Ascaris in obtaining lipid materials in hydrolysed form.

The foodstuffs available to Ascaris have no doubt been at least partly predigested by the host so that the function of the Ascaris intestine insofar as fatty acids are concerned is primarily absorptive. The cells of the foregut appear to have a secretory function, although, the enzymatic nature of the secretion has not been proved. There is nowhere in the intestine any evidence that the cells are phagocytic or that the food vacuoles characteristic of intracellular digestion are ever formed (Fairbairn, 1957).

Except for the work done by King(1966) there is very little

information concerning the movement of lipid across the gut of Ascaris, although there are strong indications which point to a very efficient lipid transport system in these nematodes. The purpose of this investigation was to study the effects of bile salts on the movement of lipid across the gut of Ascaris.

CHAPTER II

MATERIALS AND METHODS

Preparation of Mucosal Solutions:

1) Fatty Acid-Hemolymph Complex: This complex was prepared by a slight variation of the method described by Johnston (1958). 0.2 muMolar palmitic acid-1-C^{14} (36.6 mc/mM) was dissolved in a small amount of ethyl ether and mixed with four ml. of 95% ethanol. The solution was adjusted to pH8 with 0.1N NaOH and taken to dryness in vacuo at 40° C. Five ml. of hemolymph was added to the flask and the mixture was shaken mechanically for one hour at 37° C. The solution was then vacuum filtered through Whatman GF/A glass paper. The above preparation had a palmitic acid-1-C^{14} final concentration of 0.2 muMolar.

2) Fatty Acid-Albumin Complex: This preparation is similar to that of the fatty acid-hemolymph complex, except that instead of adding five ml. of hemolymph, five ml. of a 0.05% albumin solution is added. To this solution two and a half ml. of hemolymph was added, plus an appropriate amount of dextrose (7.806 mg/ml of solution).

3) Bile Acid-Ascaris saline Complex: Preparation of this complex is similar to the above two preparations, except here five ml. of a bile acid solution was added after the drying step. The dried material seemed to dissolve quite readily in the bile acid suspension. The bile acid solution was prepared by adding 19.6 mg. of Chenodesoxycholic

Acid (5β-Cholanic Acid-3a, 7a-diol) to five ml. of Ascaris saline, followed by homogenization. This solution was then designated as "saturated bile acid" and had a concentration of.01 Molar. It was necessary to homogenize the bile salt solution to enhance the solubility. This step produced a fine suspension.

Preparation of Serosal Bathing Media:

Two types of media were prepared which both contained five ml. of Ascaris hemolymph. One type contained .0786 g, of glucose and the other did not, depending on whether the experimental design required an energy source. The addition of glucose to both the bathing media and the serosal solution give final concentration of 0.043 Molar, and assured equal-molar concentrations on both sides of the gut preparation.

Preparation of the Tissue:

Adult female Ascaris Lumbricoides suum were obtained from Wilson and Company, Oklahoma City, Oklahoma. The worms were transported to the laboratory in a saline solution (Jacobson, 1965) maintained at 35° - 40° C.

The worms were sliced longtudinally and the reproductive organs were carefully removed to leave the gut tissue intact. The anterior end of the mid-gut was cut and the end of a fire polished Pasteur pipette was inserted and ligated in place with size 0 silk thread. The desired mucosal solution was injected into the gut via the pipette with the aid of a Tuberculin (1 ml.) syringe. The posterior end of the mid-gut was ligated after some of the mucosal fluid had been flushed

through, and the gut was filled. This was followed by the ligation of the anterior end of the gut just in front of the tip of the pipette. This procedure took approximately seven minutes. The resulting sac preparation was washed three times with warm saline and placed in a five ml. breaker containing half a ml. of the bathing media.

Incubation

The beaker containing the bathing media and sac preparation was placed in a water bath at 37° C. Individual beakers were covered with a glass bell jar which permitted the experiments to be carried out in atmospheres of various gases. This experiment was shaken mechanically for one hour.

Sampling and Counting:

Samples were taken of the bathing media at the start of the experiments which were designated as zero time, and at the end of each experimental time limit, which was designated as sixty minutes. Each of the samples was placed in a glass scintillation vial and evaporated under a stream of nitrogen. Ten ml. of a scintillation mixture was added and radioactivity was measured with a Packard TriCard Liquid Scintillation Spectrometer. Radioactivity of the mucosal solution was determined for each experiment. Quenching was checked on each sample by the Channels Ratio Method described by Herberg (1964).

Thin Layer Chromatography:

The bathing media, bile acid complex and the gut tissue from twelve

experiments were pooled in three separate test tubes and extracted with 15 ml. of chloroform methanol (2:1). These three samples were then taken through an exptraction described by Folch, et.al. (1951) to remove the lipids. The isolated lipid was adjusted to five ml. with chloroform, transfered to screw cap vials and stored at 4° C until utilized. Each sample was spotted on thin layer plates made with Silica Gel G and developed in hexane:ether:acetic acid (60:40:1). The components on the plates were visualized under iodine vapor. Each of the visable spots was eluted from the plates, placed in scintillation vials and counted to determine the radioactivity.

Gas Liquid Chromatography:

Methyl esters of fatty acids from the mucosal solution, gut and hemolymph bathing media were prepared and analyzed by gas chromatography techniques. The esters were prepared following standard methods which employ diazomethane. A Barber-Coleman Selectra 5000 gas chromatography instrument equipped with an ionization detector and employing argon as the gas carrier was used for the fatty acid analysis. A six foot glass column packed with 14.5 percent ethylene glycol succinate and Cas-Chrom CLP, 100-120 mesh was employed. The conditions for analysis were: column temperature 175° C; detector temperature 170° C; injector temperature 250° C. The gas-flow rate was 200 ml/min. Fatty acid esters were identified by comparison with standards. The system was equipped with a Packard gas chromatography fraction collector which allowed one to collect individual esters as they were eluted from the column. This apparatus was employed to determine the distribution of radioactivity in the fatty acids from each system analyzed.

CHAPTER III

RESULTS

Bile Salts

In order to determine which bile salt concentration would be the most effective in promoting movement of palmitic acid across the gut it was necessary to propare serosal solutions of varying bile salt concentrations. Six solutions were prepared containing 2.4, 24, 28, 100, 500 and 10,051 μMolar concentrations of Chenodesoxycholic Acid (M.W. 392.56). Figure 5 presents the results of varying the bile acid content of the experimental system. It can be seen that the movement of palmitic acid-1-C^{14}, measured in dpm/sq.cm./hr., reached a peak and leveled out at a concentration of 100 μMolar. There was great similarity of activity obtained at these higher concentrations, indicating that the system had reached its outer limit of movement of palmitic acid-1-C^{14} under the existing conditions.

To establish exactly what effect bile salts have on the system it was necessary to compare the movement of palmitic acid-1-C^{14} under similar conditions with the exception of eliminating bile salt from one of the experimental groups. Table III represents the results of three sets of experiments carried out in this way.

In summation of this section then it can be seen that bile salt concentrations of 100 μMolar or greater shows a plateau in movement.

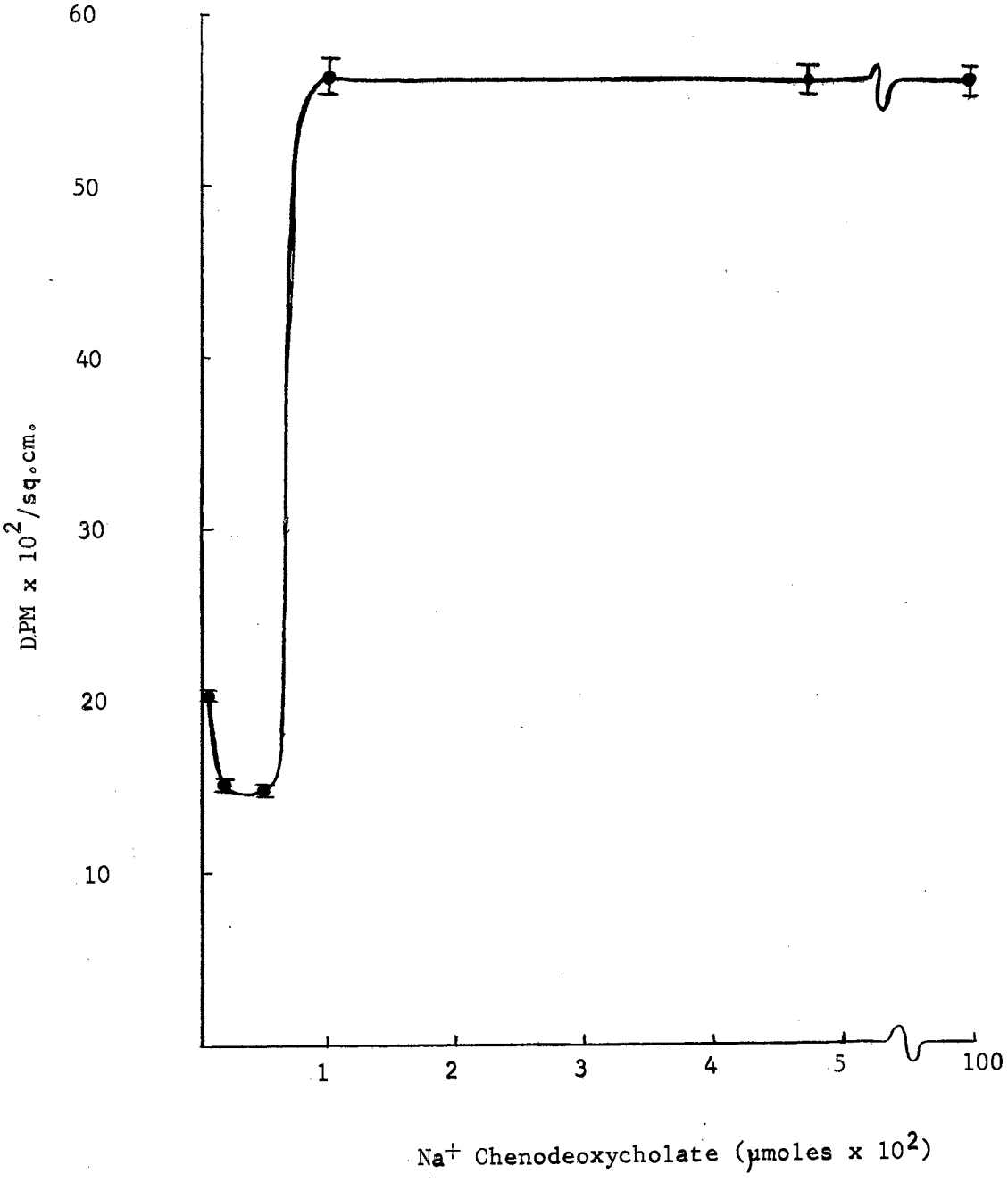

Figure 5. Effect of Bile Salt Concentration on the Movement of Palmitic Acid-1-C^{14} Across Ascaris Midgut

TABLE III

EFFECT OF PRESENCE OR ABSENCE OF BILE SALT
ON VARIOUS SYSTEMS

conditions	dpm/sq.cm./hr.	mμM/sq.cm./hr.	std.dev.
complete*	5497 (12)	8.21	± .37
complete minus salt biles	344 (3)	.51	± .049
albumin complex	1334 (4)	1.99	± .95

( ) indicates number of observations
* complete system contains "saturated" bile salt solution, 0.2 mμMolar palmitic acid, pH 8, 95%N_2-5%CO_2

When bile salts are being used there is approximately 16 times more movement that when it is eliminated from the system.

Palmitic Acid Concentration Studies

In order for the system to operate at maximum efficiency it was necessary to find a palmitic acid concentration which would produce optimum conditions for movements. Eight separate concentrations were investigated ranging from 0.1 mμMolar up to and including 30 muMolar. The variation in concentrations was obtained with 0.1 Molar palmitic acid while the concentration of palmitic acid while the concentration of palmitic acid-1-C^{14} remained the same. The results of this group of experiments are presented in Figure 6. Movement of palmitic acid-1-C^{14} is expressed in mμMoles/sq.cm./hr., which under the experimental conditions is the more accurate way of expressing activity due to the dilution factor involved in the preparation of each solution. Greatest movement was achieved when a concentration of 20 mμMolar palmitic acid was used. There is a leveling off of the curve at this concentration which would seem to indicate that the system has reached its saturation point. This may well be due to the solubility characteristics of palmitic acid in the system. At the concentration of 4 mμMolar there is a fluctuation in the linear progression of the other plotted values and when considering the congruity of the rest of the data it would be safe to assume this to represent experimental error.

pH Studies

In a series of preliminary studies the results suggested the pH

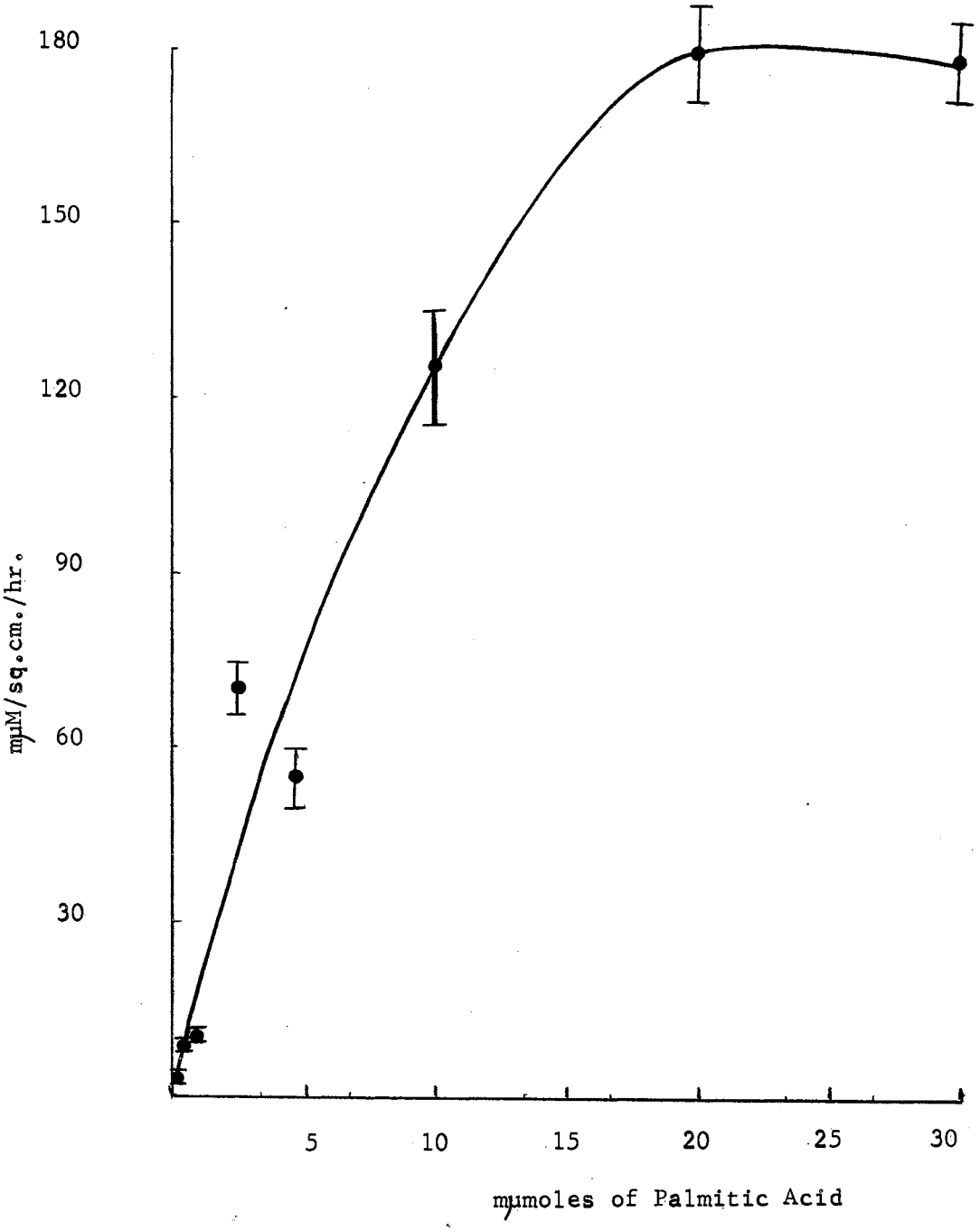

Figure 6. Effect of Various Concentraions of Palmitic Acid on the Transport of Palmitic Acid-1-C^{14} Across the Gut of Ascaris

could be a significant factor in the rate of movement of palmitate across Ascaris gut. With this information in mind a series of experiments were designed and carried out to determine the effect of pH upon the system. The pH was varied from 6.3 to 9 including 7, 7.5 and 8. The results are presented in Figure 7. The greatest activity was found at a pH of 8, and the number of experiments performed at this pH would substantiate this range as being the most efficient.

Gas Phases

King (1966) conducted a series of experiments in which the gas phase was varied. His results suggested the atmosphere might effect the rate of movement of palmitic acid, however this fact was not investigated in detail. In an effort to determine the influence of the gas phase on the movement of palmitic acid, experiments were conducted in atmospheres of 95%N_2-5%CO_2, air, N_2 and 95%O_2-5%CO_2. Except for the change in the gas phase the experimental procedure remained the same. The results of these experiments are presented in Table IV. The greatest movement was observed under anaerobic conditions. It can be seen that the presence of 5% CO_2 enhances the movement of palmitic acid-1-C^{14}. In the experiments where air and 95%O_2-5%CO_2 were used the activity decreases sharply. The rate of movement in air is one sixth the rate of 95%N_2-5%CO_2 but it is almost twice the rate of 95%O_2. Commercial gas mixtures of 95%N_2-5%CO_2 contain small quantities of oxygen. In order to determine the effect of this small amount of O_2 upon the movement of palmitic acid, the gas was passed over copper turnings heated to 400° C. The results from this experiment are tabulated in Table IV. There was a profound increase in

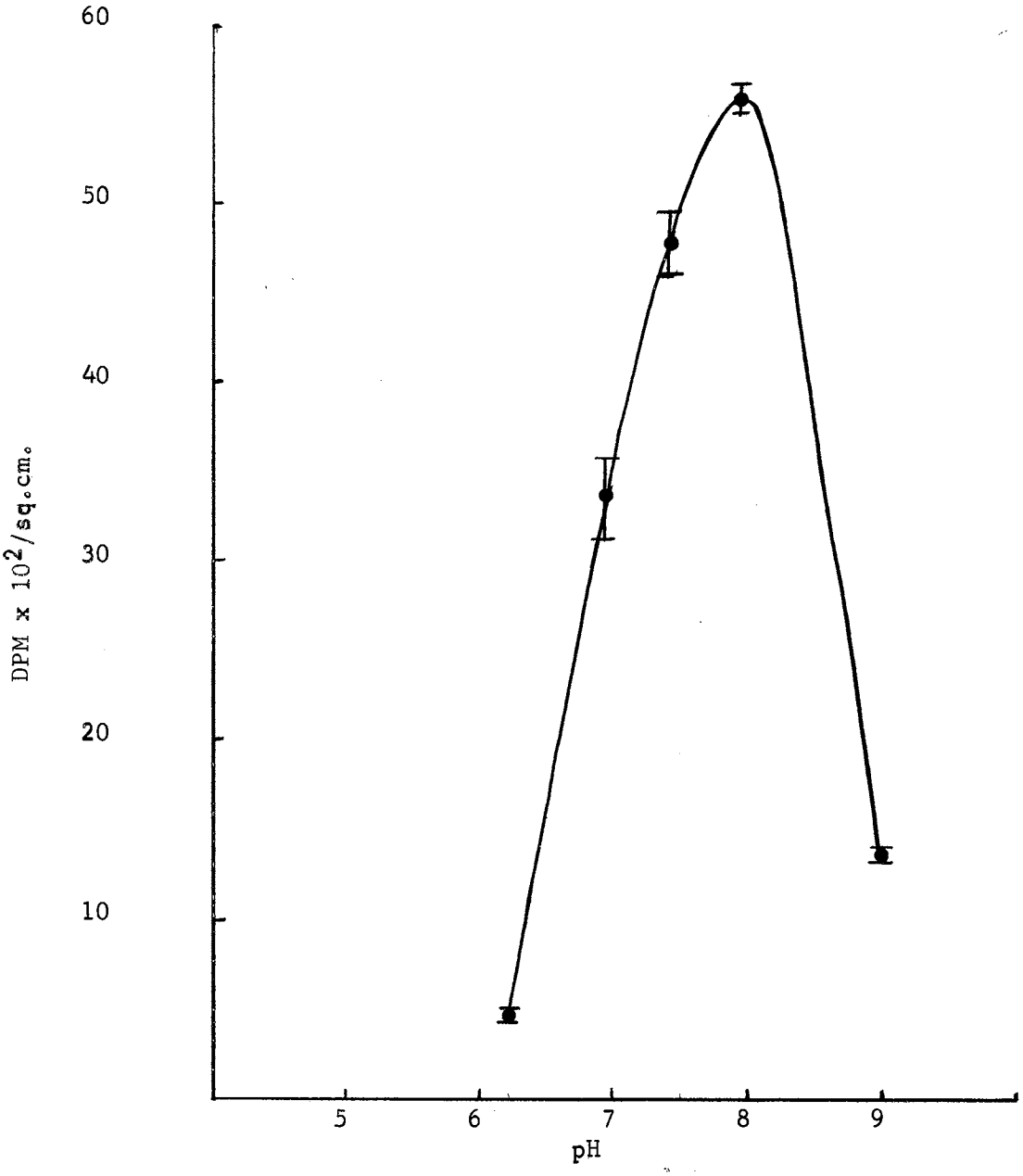

Figure 7. Effect of pH on the Movement of Palmitic Acid-1-C^{14} Across the Gut of Ascaris

TABLE IV

EFFECT OF VARIOUS GAS PHASES ON THE MOVEMENT OF PALMITIC ACID-1-C^{14} ACROSS THE GUT OF ASCARIS

gas phase	dpm/sq.cm./hr.	mμM/sq.cm./hr.	std. dev.
air	858 (3)	1.28	± .011
N_2	2213 (3)	3.30	± .0028
95%N_2-5%CO_2	5445 (9)	8.63	± .37
95%O_2-5%CO_2	459 (3)	.69	± .0028
95%N_2-5%CO_2 (heated)*	10,567 (3)	15.78	± .577

( ) indicates the number of abservations

* Gas passed over copper turnings heated to 400° C. to remove traces of O_2 in the commercial gas mixture.

the activity when the traces of oxygen was removed from the gas. This suggests that the cells of Ascaris gut are extremely sensitive to oxygen.

Energy Studies

To determine the need for an energy source in the system a series of experiments were designed with and without added glucose. Measurements were made in three gaseous phases ($95\%N_2$-$5\%CO_2$, air, and $95\%O_2$-$5\%CO_2$) and the results are presented in Table V. The greatest movement was found to occur under anaerobic conditions ($95\%N_2$-$5\%CO_2$) with 8.63 mμMoles of Palmitic acid-1-C^{14} moving per sq.cm. per hour. When the source of energy (0.043M Glucose) was removed from the system the rate of movement fell approximately 84 percent. When the experiments were conducted in air the activity was low, but if glucose was present in this aerobic system the movement was 1.4 times greater than when it was ommited. Activity recorded in the $95\%O_2$-$5\%CO_2$ system was again low, but when glucose was added to the system the activity was greater that when it was absent.

Thin Layer Chromatography

To obtain information on the mechanisms of movement of palmitic acid across Ascaris midgut the serosal solution, mucosal solution and gut tissue from three experiments were combined. Lipids were extracted seperately from the bulked samples with chloroform:methanol (2:1) and aliquots were taken for separation and identification by thin layer chromatography. Individual components from each lipid extract were removed from the thin layer chromatography plates and analyzed for radioactivity. The results of these analyses are presented in Table VI.

TABLE V

MOVEMENT OF PALMITIC ACID-1-C^{14}
ACROSS ASCARIS MIDGUT

	muMoles/sq. cm./hr.	
gas phase	with glucose	without glucose
95%N_2-5%CO_2	8.63 ± 0.37 (9)	1.45 ± 0.017 (3)
air	1.28 ± 0.011 (3)	0.92 ± 0.0057 (3)
95%O_2-5%CO_2	0.69 ± 0.0028 (3)	0.54 ± 0.0005 (3)

( ) indicates number of observations
Mucosal Fluid - 0.5 ml. Ascaris hemolymph plus 0.043M glucose
Serosal Fluid - "saturated" Chenodesoxycholic acid in Ascaris saline plus 0.043M glucose, pH 8

TABLE VI

SUMMARY OF CHROMATOGRAPHIC STUDIES

Extract	Bile Acid	Gut	Bathing Media
Compound			
Free fatty acids	81.8%	13%	93%
Triglycerides	2.0%	14%	0%
Phospholipids	3.4%	17%	3.9%
Unknown (monoglyceride)		26%	
Others*	13.8%	30%	3.1%

Solvent System: Hexane:Ether:Acetic Acid (60:40:1)

* This includes; Sterolester, Ascaroside ester, Cholesterol and unknowns.

The greatest concentration of radioactivity in the bathing media was found in the form of free fatty acids (93%) with a much smaller amount in the phospholipids (3.9%). No activity was noted for triglycerides. It was apparent from the chromatogram of the gut tissue that there was no single compound which contained a major portion of the activity. The activity was widely spread throughout all the compounds present. As was expected, almost all of the activity in the bile acid extract was found in the form of free fatty acids since in the preparation of this media palmitic acid was added as the major source of C^{14} activity.

Gas-Liquid Chromatography

It is possible that palmitic acid could be metabolized to some new acid as it is moved across the gut. For this reason it was necessary to determine exactly which acids were present in the gut tissue and in the bathing media extracts. An aliquot of each sample was carried through saponification and methylation steps preparing them for analysis by gas-liquid chromatography. This analysis determined the fatty acids present and the percentage of radioactivity each possessed. The results from this study are presented in Table VII. It can be seen that the largest amount of activity is found in Palmitic acid, 95% and 97% in the gut extract and bathing media extract respectively. Much smaller amounts of activity were found in stearic acid in both samples. All of the other fatty acids present contributed only negligable amounts of activity. When a sample of the bile acid solution was extracted and analyzed in the same manner it was found that 98% of its activity was found in the form of palmitate which had been added during its preparation.

TABLE VII

ANALYSIS OF THE GUT EXTRACT AND BATHING MEDIA BY GAS-LIQUID CHROMATOGRAPHY

Molecular Formula	Fatty Acid	% Radioactivity	
		Gut Extract	Bathing Media
$C_{12}H_{24}O_2$	Lauric	0.0	-
$C_{14}H_{28}O_2$	Myristic	0.4	0.0
C_{15}	Unknown	0.15	0.0
$C_{16}H_{32}O_2$	Palmitic	95.4	97.63
$C_{16}H_{30}O_2$	Palmitoleic	0.0	0.3
$C_{18}H_{36}O_2$	Stearic	2.4	1.5
$C_{18}H_{34}O_2$	Oleic	0.73	0.0
$C_{18}H_{32}O_2$	Linoleic	0.43	0.2
C_{20}	Unknown	0.18	0.0
$C_{18}H_{30}O_2$	Linolenic	0.17	-

(-) not indicated on the chromatogram

CHAPTER IV

DISCUSSION

The purpose of the five experiments carried out in this study was to determine the effect of bile salts upon the movement of palmitic acid across the gut of Ascaris. Some of the factors effecting movement of nonvolatile acid were investigated. In the presence of 100 μMolar bile salt the rate of movement was sixteen times the rate observed when the salts were ommitted. Comparing the movement obtained when using an albumin complex to that of the bile salts or micellar solution, the results showed that there was four times more movement with the bile salt preparation. These results are consistent with Johnston & Borgstrom (1963) who found that the uptake of fatty acid and monoglycerides was much faster from a micellar state than from an emulsified or albumin-bound state. It is evident from the results that the presence of bile salts enhances the movement of palmitic acid across the gut of Ascaris.

Examination of the results in the experiments on palmitic acid concentration optimum shows a decrease in dpm/sq.cm. when the palmitic acid concentration (in μmoles) increases. The reason for this is not a decrease in the activity but is a matter of dilution. Rate of movement, in fact, increases and is represented as μmoles of palmitic acid transported. In preparation of the 30 μmolar solution 25 μl of palmitic acid-1-C^{14} was added along with 1.5 ml. of 0.1 M palmitic acid,

whereas in the preparation of the 0.1 mμM solution only 5 ul of the 0.1 M palmitic was added to 25 μl of radioactivity. Thus there is a dilution of the labled palmitate molecules in the 30 mμM preparation. Since the detection of movement is dependent on radioactivity it can be seen that although there maybe many molecules being transported only a few will have a label and it is only these molecules that will be detected. In order to rectify this problem the activity was expressed as mμM/sq.cm./hr. which accounts for the dilution factor, rather than dpm/sq.cm./hr. which does not.

The results indicate that the gas phase in which Ascaris metabolizes best is an oxygen free system. The rate of movement or radioactivity across the gut was reduced drastically in the presence of oxygen. These results are in agreement with Harpur (1962) who found that air or oxygen had an inhibiting and even a deleterious effect upon the tissue of Ascaris. He suggests that nitrogen be used as the gas phase. Bueding et.al. (1961) pointed out that this nematode is not dependent on aerobic metabolism and in an atmosphere which contains 2%-5% CO_2 in nitrogen it survives for considerably longer periods and metabolizes carbohydrates at a higher rate than under aerobic condition. It can be seen in Table IV that the presence of CO_2 does enhance movement, in this case there was an increase of 2.5 times the activity when 5% CO_2 was added to the system. This marked effect of CO_2 in increasing the period of survival and in enhancing the rate of uptake may be due to a mechanism for CO_2 fixation. This fixation reaction is necessary to reoxidize the reduced NAD (nicotinamide-adenine-dinucleotide) which is formed during glycolysis. Reoxidation of this cofactor in the mammalian system is accomplished through the TCA cycle. This cycle seems to play no part in Ascaris metabolism,

therefore, there must be other pathways present to facilitate this reoxidation. Ascaris is able to bring about this reoxidation, anearobically, by the fixation of carbon dioxide into phospho-enod-pyruvate which produces fumarate (Saz & Vidrine, 1959; Saz, personal communication).

When glucose was ommitted from the medium there was a decrease in the movement of radioactivity across the gut wall. Table V illustrates this point even to the point of showing a decrease in activity in a system ($95\%O_2$-$5\%CO_2$) which has a very low rate of movement even with glucose. In each of the systems listed there was a marked decrease in activity when glucose was ommitted, thus the presence of glucose, or the presence of an energy source does enhance the movement of palmitic acid-1-C^{14} . This would suggest that there is an energy dependent mechanism associated with the movement of fatty acids in this nematode. Johnston & Borgstrom (1963) found that fatty acids and monoglycerides from micellar solutions were found to be incorporated quickly into triglycerides by an enzymatic and energy dependent process. The carbohydrate metabolism of Ascaris proceeds by way of the Embden-Meyerhof pathway, with only part of the TCA cycle enzymes being present (Beuding, 1962). Thus it is likely that most of the metabolic energy of this nematode comes from reactions occuring in glycolysis.

When the results from all five sections of this study are considered it can be seen that an energy source and an anaerobic environment are two factors that have a definite effect on the movement of palmitic acid-1-C^{14} across the gut wall. With these two factors, and knowing that the system is affected by pH and that saturation at higher concentrations of 0.1M palmitate does occur would fulfill the kinetic requirements of a mechanism involving metabolic pathways in the movement

of fatty acids across the gut.

When the distribution of radioactivity in the bathing media and gut tissue was determined it was found that 93% of the activity of the bathing media was found in the free fatty acids, whereas in the gut tissue the fatty acids accounted for only 13% of the total activity. In the analysis of the bile extract approximately 82% of the activity was in the form of free fatty acids. This suggests that the gut cells take up the fatty acid and then subject them to a series of steps in which they are transformed into other compounds including triglycerides, phospholipids, sterol esters and possibly monoglycerides. There are two possibilities to explain the high percentage of free fatty acids found in the bathing media: one is that the fatty acids are moved into the gut, esterified and formed into chylomicrons. Chylomicrons then move into the bathing media (hemolymph) where they are broken up by lipases. The other possibility is that the fatty acids are never esterified in the gut but move through the cell and into the hemolymph. At the present time there is not evidence to support either one.

CHAPTER V

SUMMARY AND CONCLUSIONS

Experiments have been designed and carried out to determine the effect of bile salt on the movement of palmitic acid across the intestinal wall of Ascaris. Factors effecting movement such as substrate concentration, pH, energy requirements, and gas phases were investigated. Lipids of the bathing media and gut tissue were analyzed and the distribution of the radioactivity was determined. From the data obtained, the following summary and conclusions can be made.

1. When bile salts were present in the system, the movement of palmitic acid across the intestinal wall was enhanced. The greatest degree of activity was obtained using a concentration of 100 μMolar.

2. Best results were obtained when palmitic acid concentrations of 20 mμmolar were used.

3. When the pH of the system was adjusted to 8 movement across the gut was enhanced.

4. When an energy source was added to the system, the movement of palmitate across the gut increased significantly.

5. Movement was best in an oxygen free system. The highest rate of movement was observed when 5% carbon dioxide was present in the gaseous mixture.

6. The bulk of the radioactivity in the bathing media was in the form

of free fatty acids, and 98% of it was found in palmitic acid. In the gut tissue radioactivity was distributed between fatty acids, triglycerides, phospholipids and what is tentatively identified as monoglyceride.

These results suggest that there is an energy requiring mechanism present in Ascaris for the movement of palmitic acid across the intestinal wall. This mechanism may or may not be similar to that found in the mammalian small intestine.

There are many experiments that can be performed utilizing some of the techniques employed in this study. Hopefully, experiments utilizing the conjugated bile salt, glycerides and even other fatty acids will be carried out in the near future. At the present time there is a need for further study on the mechanisms concerned with the movement of these lipid moieties.

BIBLIOGRAPHY

Baker, R. D., and G.W. Searle. "Absorption of Dietary Lipids in the Rat Intestine." Proc. Soc. Exptl. Biol. Med. Vol. 105. (1960) 521-543.

Beames, Calvin, G.,Jr., Ben G. Harris, and Fred A. Hopper. "The Synthesis of Fatty Acids from Acetate by Intact Tissue and Muscle Extract of Ascaris lumbricoides suum." Comp. Biochem. Physiol. Vol. 20. (1967) 509-521.

Borgstrom, B., G. Lundh, and A. Hofmann. "The Site of Absorption of Conjugated Bile Salts in Man." Gastroenterology. Vol.45. (1963) 229-246.

Brown, J.L., and J.M. Johnston. "Radioactivity of Lipid Components Separated by Thin-Layer Chromatography." J. Lipid Res. Vol. 3. (1962) 480-491.

Bueding, E., "Comparative Aspects of Carbohydrate Metabolism." Fed. Proc. Vol. 21. (1962 1039-1046.

Bueding, E., E. Kemetec, S.A. Swartzwelder, and H.J. Saz. "Biochemical Effects of Dithiazine on the Canine Whipworm Trichuris vulpis." Biochem. Pharmacol. Vol. 5. (1961) 311-322.

Carpenter, M.F. "The Digestive Enzymes of Ascaris lumbricoides suum: Their Properties and Distribution in the Alimentary Canal." Ph.D. Dissertation, Univ. of Michigan, Detroit, Michigan (1952).

Dawson, A.M., and K.J. Isselbacher. "Esterification of Palmitate-1-C^{14} by Homogenates of Intestinal Mucosa." J. Clin. Invest. Vol. 30. (1960) 156-160.

Fairbairn, D. "The Biochemistry of Ascaris." Exptl. Parasitol. Vol. 6. (1957) 491-554.

Fairbairn, D., and R.F. Passey. "Occurrence of Distribution of Trehalose and Glycogen in the Eggs and Tissues of Ascaris lumbricoides." Exptl. Parasitol. Vol. 6. (1957) 566-674.

Feldman, E.B., and B.Borgstrom. "Intestinal Absorption of Lipids." Lipids. Vol. 1. (1966) 128-131.

Florkin, M., and E.H. Stotz. "Bile Salts." In Comprehensive Biochemistry. Vol. 10. Elsevier Publishing Co., New York. (1963) 23-31.

Folch, J., I. Ascolf, M. Lees, J.A. Meath, and F.N. LeBaron. "Preparation of Lipide Extracts from Brain Tissue." J. Biol. Chem. Vol. 191. (1951) 833-841.

Guyton, A.C. "Lipid Metabolism." In Textbook of Medical Physiology. W.B. Saunders Company, Philadelphia. (1964) 889-903.

Harpur, R.P. "Maintenance of Ascaris lumbricoides in Vitro. A Biochemical and Statistical Approach." Can. J. Zool. Vol. 40. (1962) 991-1011.

Hartley, G.S. Progress in the Chemistry of Fats and Other Lipids. Vol.III. Pergamon Press, London. (1955) 19-29.

Herberg, R.J. "Channels Ratio Method of Quench Correction. In Liquid Scintillation Counting." Technical Bulletin No. 15 (1964) 1-7.

Hofmann, A.F., and B. Borgstrom. "Physico-chemical State of Lipids in Intestinal Content During Their Digestion and Absorption." Gastroenterology. Vol. 21. (1962) 43-50.

Jacobson, N.S. "Lipid Metabolism in Female Ascaris lumbricoides During Starvation." Ph.D. Dissertation, Oklahoma State Univ., Stillwater, Oklahoma (1965).

Johnston, J.M. "An In Vitro Study of Fatty Acid Absorption." P.S.E.B.M. Vol. 98. (1958) 836-838.

Johnston, J.M., and B. Borgstrom. "The Intestinal Absorption and Metabolism of Micellar Solutions of Lipids." Acta. Chem. Scand. Vol. 17. (1963) 905-919.

Kessel, R.G., J.J. Prestage, S.S. Sekhon, R.L. Smalley and H.W. Beames. "Cytological Studies on the Intestinal Epithelial Cells of Ascaris lumbricoides suum." Trans. Amer. Micr. Soc. Vol. 80. (1961) 103-118.

King, G.A. "Transport of Palmitic Acid Across the Gut of Ascaris lumbricoides suum." M.S. Thesis, Oklahoma State Univ., Stillwater, Oklahoma. (1966).

Lack, L., and I.M. Weiner. "Intestinal Absorption of Bile Salts and some Biological Implications." Fed. Proc. Vol. 22. (1963) 1334-1338.

Oser, B. "Bile Salts." In Hawk's Physiological Chemistry. 14th. Ed. McGraw-Hill Book Company, New York. (1965) 488-501.

Read, C.P. "The Vertebrate Small Intestine as an Environment for Parasitic Helminths." Rice Institute Pamphlet. Vol. 37. (1950) 1-94.

Reiser, R., and C.H. Fu. "Acyl Group Exchange in the Intestinal Lumen During Fat Digestion." Biochim. Biophys. Acta. Vol. 116. (1966) 563-569.

Rogers, W.P. "Digestion in Parasitic Nematodes I. The Digestion of Carboydrates." J. Helminth. Vol. 18. (1940) 143-154.

Rogers, W.P. "Digestion in Parasitic Nematodes II. The Digestion of Fats." J. Helminth. Vol. 14. (1941) 35-46.

Saz, H.J., and A. Vidrine. "The Mechanism of Farmation of Succinate and Propionate by Ascaris lumbricoides Muscle." J. Biol. Chem. Vol. 234. (1959) 2001-2005.

Spencer, R.P. "Spatial Distribution of Intestinal Activities." Yale J. Biol. Med. Vol. 36. (1964) 279-294.

White, A., P. Handler, and E.L. Smith. "Lipid Metabolism." In Principles of Biochemistry. McGraw-Hill Book Company, New York. (1964) 429-462.

APPENDIX A

THE EFFECT THAT VARYING BILE SALT CONCENTRATIONS HAS ON MOVEMENT OF PALMITIC ACID-1-C^{14} ACROSS THE GUT OF ASCARIS

Bile Salt Conc. (μM)	dpm/sq.cm./hr.		mμM/sq.cm./hr.	std.dev.
2.4	2020	(3)	3.07	± .176
24	1540	(2)	2.30	± 0.00
48	1512	(3)	2.26	± .023
100	5653	(3)	8.44	± .631
500	5481	(3)	8.19	± .287
"saturated (10,051)	5497	(12)	8.21	± .370

( ) indicates number of observations
Serosal Fluid- varying con. Chenodesoxycholic Acid, .2 mμMolar palmitic acid, pH 8, .043M Glucose
Mucosal Fluid- Ascaris hemolymph, .043M Glucose
Experimental Time- one hr.
Temperature-37° C.
Atmosphere- 95%N_2-5%CO_2

APPENDIX B

EFFECT OF PALMITIC ACID CONCENTRATION ON THE MOVEMENT OF PALMITIC ACID-1-C^{14} ACROSS THE GUT OF ASCARIS

Palmitic Acid Con. (mμMoles)	dpm/50μl	dpm/sq.cm.	dpm/μl	dpm/mμM	mμM/sq.cm./hr	std. dev.
.1	8902	4486	178	1780	2.52	± .003
.2	6695	5658	133.9	669.5	8.45	± .370
.5	6065*	2515	121	242	10.39	± .980
2	3925	2793	78.5	39.3	71.06	± 5.08
4	6246	1734	124.9	31.2	55.57	± 8.25
10	4651	1179	93	9.3	126.77	± 12.13
20	6974	1252	139.5	6.9	181.44	± 10.14
30	6336	754	126.7	4.2	178.67	± 6.53

* This value was obtained by averaging the other values in the column.
Serosal Fluid- "saturated" Chenodesoxycholic Acid, varying con. of palmitic acid, .043M Glucose, pH 8.
Mucosal Fluid- Ascaris hemolymph, .043M Glucose
Experimental Time- one hr.
Temperature- 37° C.
Atmosphere- 95%N_2-5%CO_2

APPENDIX C

THE EFFECT OF pH ON THE MOVEMENT OF PALMITIC ACID-1-C^{14} ACROSS THE GUT OF ASCARIS

pH	dpm/sq.cm./hr.	mμM/sq.cm./hr.	std.dev.
6.3	464 (6)	.69	± .059
7	3371 (3)	5.0	± 1.82
7.5	4790 (3)	7.2	± 1.96
8	5581 (12)	8.3	± .370
9	1328 (3)	2.0	± .068

( ) indicates the number of observations
Serosal Fluid- .2mμM Palmitic Acid, "saturated" Chenodesoxycholic Acid, .043M Glucose, varying pH
Mucosal Fluid- Ascaris Hemolymph, .043M Glucose
Experimental Time- one hr.
Temperature- 37° C.
Atmosphere- 95%N_2-5%CO_2

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