FECAL LOSSES OF LIPIDS:

The influence of dietary fat and of bile deprivation in dogs

by

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INTRODUCTION

The general topic of fat assimilation has intrigued man for over a century. His recorded investigations up to 1932 have been well documented in the Monograph of Physiology, edited by Verzar and Lastz, entitled "Absorption from the Intestine." (60). In this monograph these authors quote from work completed independently by Claude Bernard and Dastre near the end of the Eighteenth Century. These works consist of recorded observations made on the intestinal lymphatics of animals in an absorptive state. Claude Bernard first observed in rabbits that milky chyle was seen in the intestinal lymphatics only below the entrance of the pancreatic duct. In this animal the bile duct enters the intestine some thirty centimeters proximal to the pancreatic duct entrance. This observation was made more meaningful by Dastre, who divided the common bile duct and anastamosed the gall bladder to a lower segment of the small intestine in rabbits. He observed in these rabbits that the chyle of the intestinal lymphatics was milky only below the point of gall bladder anastamosis. The work of these two authors led Verzar to conclude that both bile and pancreatic juices were necessary for fat absorption.

It was many years before another investigation inquiring into the basic question of fat absorption was undertaken. The paucity of any study concerned with fat absorption was completely overshadowed by the developments focused around

the physical characteristics of the lipids as well as the bile. This has been well documented by Sobotka (48) and Deuel (21).

The physical properties of triglycerides and fatty acids differ markedly. Several important parameters include melting point, saturation, chain length, boiling point and specific gravity.

In view of the differences, which in these respects may be said to characterize the fats to some degree, one should not be surprised that the early investigations showed that fats of differing "composition" differed widely in terms of their assimilation from the gastrointestinal tract of man and animals. This was documented early in Verzar's Monograph (60) under the discussion of fat absorption. Here, it was shown that olive oil, pig's fat and plant fats were extremely well absorbed (98%), while sperm oil and pure stearin were poorly absorbed (less than 15%). The significance of this difference has not been explained, nor has the mechanism of the differential absorption been worked out. This is understandable when it is considered in the light of our very limited knowledge regarding the ability of the intestine to assimilate individual fats.

The investigation of the assimilation of fat by the animal body has been approached by several different methods. The latest method developed to estimate absorption utilizes I^{131} radioactivity. This has been used by Wells (63) and the recent literature reviewed by Turner (59).

Earlier methods designed to study fat absorption employed intestinal lymphatic or thoracic duct cannulation, and estimated the amount of fat absorbed from the recovery of fats transported via this route, Also reported in the early literature was the use of isolated intestinal segments, either as blind pouches or as loops of small bowel, to quantitate some aspects of fat absorption. However, early studies were also reported using fat balance studies, e.g. Smith et al (47). While this type of investigation is not as sensitive as some of the more selective techniques, it directly quantitates absorption through an inverse relationship to fecal fat excretion. This is in direct contrast to the other methods that have been used under limited adverse conditions to estimate absorption, but which do not truly measure absorption. Of these the lymph lipid investigations must be interpreted in terms of fat transport as an estimate of absorption, rather than absorption, per se. In this light the fat balance study, in which the dietary fat intake is known and the fat loss is measured by extraction of the fecal material, can approach absorption directly and surely. Although this type of investigation is not capable of pin pointing the mechanism of absorption it yields information quantitating fat absorption than can be obtained directly, without inference, in no other way.

REVIEW OF THE LITERATURE

It is important at the outset to introduce the concept that the small bowel is not merely a one way street of absorption, but is also an organ of secretion and excretion dealing not only with the implements of digestion, but also with the products of digestion. This has been shown to be true for fats introduced into Thiry-Vella Fistula preparations of dogs by Angevine (1). He found fat to be present in the basal secretion from these intestinal fistulae and determined that the quantitative fat excretion by these loops was not appreciably affected by the content of fat in the diet.

Other methods utilized in approaching this problem of fat excretion have included fat balance studies and thoracic duct fistula preparations of control and biliary fistula dogs. Thus, in 1930, Sperry (53) using bile fistula dogs described a two to three-fold increase in the fecal excretion of lipids following bile deprivation when the dogs received a fat free diet. Lard, when added to the fat free diet of these bile fistula dogs, markedly increased the fecal excretion of fat. Centrifugal separation of the feces in aqueous solution, and extraction in Soxhlet apparatus with petroleum ether, demonstrated that while the heavier bacterial fraction contained measurable fat, the increase in fecal fat following bile fistula preparation took place in the non-bacterial component. This work is of significance

when one considers the monumental work that Sperry and his co-workers have done to elucidate the source of fecal lipids in the dog (6,8,11,12,16,30,31,49-54,56). These investagations have shown in the normal dog that fat loading does not appreciably increase the quantitative fecal lipid excretion (56), that the ratio of liquid to solid fatty acids excreted in the feces varied directly with the type of dietary fats (49), that bile, per se, was not the source of the total lipid in feces (50), that the bacterial fraction of normal fecal material contains about 40% of the total amount of fecal lipid (52), but that in the bile fistula dog the bacterial fraction contains none of the increased lipid excretion (53). In his consideration from original calculations the lipid does not arise en toto from desquamated epithelial cells (51), and demonstrated in ileostomy dogs that the ileostomy flow has a greater fat content than the fecal content when the animals were fed a fat-free diet (56).

In 1933 Rony (40) investigated the source of lymph lipids in the fasting dog. He demonstrated that lymph fat transport was greater in the fasting state, i.e. 48 to 168 hours postprandially than 24 hours post-prandially. Following a sub-total enterectomy, performed in "fasting" animals, the thoracic duct lymph lipid content was lower than in intact fasting animals. In fasting bile fistula animals the thoracic duct lymph lipids were not significantly different in quantity than in the control fasting animal.

Following the injection of pilocarpine the lymph lipids of the intact fasting dog increased but in the fasting enterectomized preparation this manipulation failed to evoke the increase in lymph lipid content. These manipulations suggested to the author that fasting lymph lipids arise for the most part in the intestine, possibly through the continual absorption of small quantities of intraluminal lipids. It would not seem unreasonable if this continued "secretion" and absorption of fats takes place as suggested, that some of this fat would ultimately appear in the feces as endogenous fecal fat.

As it has been presented above, the concept of endogenous lipid excretion is not susceptable to a straight forward approach, and so will require a great deal of experimentation and data accumulation to elucidate its origin. This question of endogenous fat excretion was further investigated using isolated Thiry-Vella fistula loops of intestine by Peretti, as quoted by Deuel (21). He found that when he fed iodized oils to fistula animals he could recover iodized oil from the fistula secretions. These studies also give one the impression that origin of endogenous lipid excretion is one of secretion, but even though it is consistent with that hypothesis, it is not specific nor is there any adequate amount of other accumulated evidence to justify any more than a speculative conclusion.

Utilizing fat balance studies, various authors (3-5, 16,19,20,25,26,28,32,33,34,41,44,51,52-54,56,63,64,66,67) have found total fecal fat excretion in normal dogs, cats, rats, patients, and medical students to lie between about one and eight grams of fat per day. This of course varies with the size of the animal, the amount of dietary fat, and the extraction method used. Normal dogs usually reach a high of about five grams of fat per day (3,63). This fat excretion in dogs is apparently independent of carbohydrate and nitrogen excretion (4).

It should be made clear before proceeding further that the lipid substances excreted in the feces are not merely unabsorbed dietary substances. This has been shown by Holmes and Kerr (28) who demonstrated that fecal fats had a higher melting point than dietary fats, that the iodine number was lower and the saponification number was lower than dietary fats. This was substantiated in one way by Deuel (21) who showed that in the feces the ratio of solid to liquid fats differed. Also, it should be emphasized that the feces contain fat when none is ingested or during starvation periods (60).

Influence Of Bile Acids On The Loss Of Fats From Isolated Intestinal Loops

The question of the effect of bile upon the absorption of fat from the intestine has been investigated in several ways (2,13,17,35,57,38,43,50,61). The authors interested in this question used three techniques, briefly outlined in the introduction to approach the problem. These are the fat balance study, the isolated intestinal segment, and the thoracic duct fistual preparation studied in the control and in the bile deficient state.

The effect of bile upon fat absorption was first quantitatively appreciated in the early part of the Twentieth Century by O.H. Plant (37). He investigated the effect of dessicated ox bile on fat loss in Thiry-Vella jejunal fistula dogs. He noted that the addition of a 5 to 10 per cent aqueous solution of whole ox bile would enhance the absorption of cream, soap solutions of sodium oleate in a vehicle of either neutral cottonseed oil or water, but would not enhance the absorption of neutral cottonseed oil itself. Later, Riegel et al (38) also using isolated jejunal loops of small intestine, studied the influence of the bile salts on oleic acid absorption. These investigations confirmed the more general observations of Plant (37), and demonstrated that sodium taurocholate significantly increased oleic acid absorption over and above that seen in the absence of taurocholate. Furthermore, sodium taurocho-

late was as effective in this respect as either whole gall bladder bile or hepatic bile.

Another group, Virtue et al (61), investigated the influence of bile and bile salts specifically on the absorption of sodium oleate from straight blind loop jejunal fistulae in dogs. From their study they considered a significant amount of soap to be absorbed from the loop in the absence of bile. Their studies with added whole bile demonstrated that bile facilitated absorption of sodium oleate, but that sodium glychocholate or sodium taurocholate did not enhance its absorption. They thus differed from Riegel et al (38) who did not find significant absorption of the free oleic acid introduced without bile into Thiry-Vella loops. However, as Virtue et al (61) point out, the previous group of authors used the free acid while they used the more water soluble soap, sodium oleate. Further, it is of interest that Virtue et al (61) found the specific bile acids did not enhance sodium oleate absorption, while Riegel et al (38) found the specific bile acid sodium taurocholate to enhance oleic acid absorption.

Fecal Lipids In The Bile Deficient State

In 1927 Sperry (50) investigated the fecal lipids of dogs in the absence of bile and found that while ingesting a completely fat-free diet they excreted 1.5 to 4.5 times the average excretion when bile was present, as extracted with a Soxhlet apparatus using petroleum ether as the solvent. The normal mean was 1.9 grams total/week (seven days) while the bile fistula mean was 4.9 grams total/week. He further fractionated the fatty acids recovered from the feces into liquid and solid components. The ratio of liquid to solid fatty acids in the normal dog was 2.02 while in the bile deficient dogs was 1.09. This finding would tend to indicate that in the bile deficient state fecal lipids contain relatively more saturated fats than in the bile acid present state. If this difference were real, it would indicate bile to be of greater value in the absorption of the saturated fatty acids than the unsaturated type of fats. In 1930 Sperry (53) published a more extensive study of the partition of the fecal lipids in the bile fistula dog, and in 22 experiments on five such dogs, found the total lipid excretion to lie between 2.6 and 7.2 grams per seven days when fed a fat-free diet. When 35 grams of fat was added to the diet in the form of lard over the seven day period, he recovered 16 to 26 grams in their feces. When fed at the level of 14 grams total per seven days he recovered 4 grams of fat in the feces. These

feces were separated into bacterial and non-bacterial fractions in both groups of dogs. It was readily apparent from analysis of the individual fractions that the increase in fecal lipids in the bile fistula dog fed a fat containing diet was in the non-bacterial fraction and resulted from deficient absorption rather than a change in bacterial flora and a resulting change in fat content.

Another type of study was attempted in 1936 by Shapiro et al (44). These authors had two healthy patients with biliary fistulae that could be controlled with the drainage tube inlying in the common bile duct such that bile could be completely diverted from the intestine at will. They fed the patients deuterium labeled linseed oil which had properties similar to olive oil and which was added to a standard fat containing diet. When bile was permitted to flow into the intestine they were able to recover 1.2 to 2.0 grams of fatty acids from the stool compared to the 8.1 to 8.4 grams of fat fed. When bile was diverted from the intestine they were able to recover 8.1 and 9.4 grams of fecal fatty acids compared to the 8.5 and 8.1 grams fed, respectively. From the fraction of the total amount of fed deuterium that appeared in the feces they concluded that 65% to 75% of the dietary fat had been absorbed, and that the greater part of the fecal fat in the bile deficient patients had arisen from the fat secreted into the intestine. This study supports the concept that in the bile deficient animal

a marked increase in fecal lipid excretion occurs even though a significant amount is absorbed, but does not in any parameter define its origin.

In 1940 Coffey et al (17) studied in one dog the effect of common bile duct ligation on the absorption of foodstuffs including fats. They fed at three levels 108, 19, and 10 grams of fat a day. They observed at the two higher levels of fat intake that the dog excreted 47% of its fat intake. At the lowest level of fat intake it excreted only 28% of its intake. These values may be compared to studies completed in normal dogs by the same authors using the same methods. In one such series they fed 113 grams of fat per day and recovered 3.6% per day of the fed amount in the feces, and in a second series they fed 17 grams of fat per day and recovered 4.0% per day of that fed in the feces. These investigations demonstrate that in normal dogs fecal fat excretion varied between 2.1 and 4.0% of the dietary fat even in the face of fat loading. On the other hand the excretion of fat in bile deficient animals when related to the level of fat intake showed that at higher levels of intake a greater proportion of the dietary fat quantity was recoverable in the feces. These studies are in agreement with those of Heersma and Annegers (25) who studied the effect of bile fistulae in dogs in terms of the excretion of fecal fat with respect to the amount of dietary fat intake. They observed in six normal

dogs fed Pard with added amounts of lard with the total fat intake ranging from 15 to 47.5 grams per day, that there was a mean excretion of 3.51 ± 0.73 grams of total fecal fat per day. In nine bile fistula dogs they found that when fat intake increased from 0 to 51 grams per day the fecal fat increased from 2.7 to 33.3 grams of fat per day, respectively. The mean daily fat excretion in three of the bile fistula dogs fed fat free diet was 2.7 grams. These authors also call attention to the fact that in the absence of bile a considerable absorption of dietary fat takes place and is unexplained by the present theories of fat absorption. These authors also studied the effectiveness of substitution therapy on correcting the steatorrhea of biliary deficiency (27). They fed 36 grams of fat per day to cholecystonephrostomized dogs in one meal. The bile therapy was given at the same time mixed with the meals. Three grams of bile salt were also administered each day in an attempt to correct the steatorrhea. This amount was found experimentally to be the amount of bile output in a healthy fistula dog in which the bile is returned every eight hours (6). They observed that three gram doses of dessicated ox bile, precipitated ox bile, and dehydrocholic acid were completely without effect on the fecal fat excretion. They did note effectiveness with six grams of dessicated ox bile which reduced the steatorrhea by 25% and fresh ox bile equivalent to six grams dessicated ox bile, increased the degree of correction to 50%. These authors

felt the defect was inexplecible with their present appreciation of the problem.

These authors also showed that simple cholecystectomy had no effect upon the fecal fat excretion in dogs, demonstrating that alterations in the rate of normal bile flow into the intestine did not alter the gross pattern of fat absorption (26). They continued their investigation in this area and, as they had demonstrated in their previous investigations, bile fistula dogs excreted an average of 25.7 grams of fecal fat when they were fed 36 grams of fat per day.

In pursuing the correction therapy of biliary steatorrhea they changed the rate of bile administration (42) so that in four bile fistula dogs they were returning the dogs own bile, introducing it through the duodenum every eight hours. When the dogs reached a steady state of 6 to 8 grams of bile output per day, the collected bile introduced into the duodenum every eight hours was noted to reduce the steatorrhea to approximately 50% of that of the pre-bile-administering level. When the bile was introduced at more frequent intervals of every two to four hours, with no alteration in the cholic acid content per day, the fecal fat output decreased to a lower level. When the bile was returned every hour with no change in the total cholic acid quantity per day, the fecal excretion of fat returned to the control levels of intact dogs. If

the return of bile was stopped the fecal fat output increased as before. From the third to the seventh day following stoppage it averaged 17.8 grams and from the eighth to the thirteenth day it averaged 22.0 grams per day. The authors believed these findings to be consistant with the demonstration of bile salts in the mucosa of bile fistula dogs 24 hours following the construction of the fistula, but its absence in bowel mucosa thirty days following diversion of the biliary secretions (60).

Searle and Annegers (43) further investigated the correction of biliary deficient steatorrhea using various preparations of the bile salts. They found duodenal introduction of sodium taurocholate to be the most effective method of correcting this type of defect in dogs. Intra-duodenal glycocholate was shown to be nearly as effective as sodium taurocholate, while oxidized cholic acid had no corrective properties. Whole ox bile was shown to be effective, but it had to be used in large doses of twelve grams per day to approach control fecal fat excretion in the normal animal. It should be noted that the quantitative correction was the same whether 36 or 51 grams of fat were ingested each day. This would indicate that bile salts do not facilitate fat absorption in a constant proportional amount. These authors calculated the percent fat absorption from the equation below:

$$\text{Percent Absorption} = 100 - \frac{100\ (\text{total fecal fat} - \text{endogenous fecal fat})}{\text{dietary fat}}$$

This equation is based on the assumption that all of the fat appearing in the feces of intact dogs is endogenous in origin. This was based, firstly, on the observation that triglyceride loading in intact animals did not increase the quantity of fecal fat significantly, and secondly, that the endogenous fat of bile fistula animals did not differ significantly in amount from that of normal animals. The primary assumption is not entirely justified, for even though fecal fat excretion remains constant with fat loading in the normal dog, this does not give any information regarding the origin of the fecal fat. Further, it has been shown previously that on a fat-free diet the fecal lipid levels are increased in bile fistula animals over and above that excreted in intact animals (50).

Annegers (2) pursued the question of the action of bile by trying to correct biliary deficient steatorrhea with the administration of an emulsifying agent "Tween 80" and observed that it did not affect the fecal excretion of fat when the fat intake varied from 15 to 40 grams of fat per day. These results tend to indicate that the "solubilizing" role of the bile acids is not primarily concerned with fat absorption, and that bile may well have a more significant role in fat absorption per se than it does in the digestive processes as we know them.

The following year Pessoa, Kim and Ivy (35) investigated the effect of bile on fat absorption much as Annegers et al (2,25,27,42,43) and Coffey et al (17) had done.

However, Pessoa et al (35) were more interested in the absorption of individual or more specific fats than either Annegers et al or Coffey et al. Thus, when Pessoa et al (35) investigated this problem they used corn oil, the fatty acids of corn oil, derived by hydrolysis, and oleic acid rather than the mixtures of fat containing dog food and lard which had been used by the previous authors.

In their studies on bile fistula animals they observed the endogenous fat excretion to rise from 39.4 $\pm$ 1.7 milligrams per kilograms body weight per day (mgm./Kg./D) in normal animals to 142.2 $\pm$ 32.3 mgm./Kg./D in bile fistula animals, when fed a fat-free diet. This is in distinct contrast to Searle et al (43) who assumed that the endogenous fat excretion in normal and bile fistula animals was not significantly different. These authors support the observations of previous investigations (2,17, 25,27,42-44,53) that substantial amounts of fat are absorbed from the intestine in the absence of bile acids. In the normal dog 93% of the oleic acid fed was absorbed as compared to 70% absorption in the bile fistula dog. This contrasts sharply to the corn oil fatty acids, which by composition contain greater than 80% fatty acids, in which 89% was absorbed in the normal state compared to 55% absorption in the bile fistula animal. The authors considered the difference in percentage absorption in the bile fistula animal when fed oleic acid versus hydrolyzed corn oil to

be not significant. There was no significant difference between fatty acid absorption and triglyceride absorption in the bile fistula dog. By their calculations the endogenous fecal fat excretion in normal dogs lay between 0.188 and 0.501 grams per day with a mean value of 0.366 grams per day.

Siperstein (46) and his co-workers have contributed in a different manner to the study of fat absorption by investigating the lymphatic transport of palmitic acid, labeled in the carboxyl grouping with radio active C^{14} in intact and bile fistula rats.

These authors observed a marked reduction in percent lymph transport of the administered C^{14} labeled palmitic acid in bile fistula rats as compared to the normal controls. They found a 34% transport of the administered label in the controls, but were able to recover only 2% to 7% of the administered label in the bile dificient animals. They interpreted these transport studies as presenting evidence for decreased absorption of palmitic acid-1-C^{14} in the bile deficient rats. It should be noted, however, that the direct observation made was a marked defect in the lymphatic transport of C^{14} label.

If this above study is contrasted with a later study accomplished by Borgstrom (13), it is quite evident that transport and absorption are two distinct and different phenomena of fat assimilation. Borgstrom (13) also studied

the assimilation of palmitic acid, using C^{14} located in the carboxyl position of palmitic acid, in control and bile fistula rats. He also noticed a marked reduction in the amount of C^{14} recoverable from the lymph of bile fistula animals. However, he investigated the phenomenon further and assayed the disappearance of C^{14} radioactivity from the intestine.

Siperstein (51):

Lymph transport of fed lipid

Normal	34%
Bile deficient	2% to 7%

Borgstrom (23):

Percent of C^{14} fed that disappeared from the G.I. tract

Normal	87%
Bile deficient	65%

Percent of absorbed C^{14} transported via the lymph

Normal	66.5%
Bile deficient	19.5%

As the data is presented in tabular form above, it is easily seen from Borgstrom (13) that the amount of fat absorbed, i.e. disappeared from the intestine, can not be estimated from the amount of fat transported via the lymph. However, it is important that in these two investigations the defect in fat transport via the lymphatics in the bile dificient animals as measured with C^{14} labeled palmitic acid, is of the same order of magnitued, quantitatively

about 10% of that administered, as compared to fat transport via the lymphatics in the control animal of about 30% to 50% of that administered.

Borgstrom (13) was also able to show in the control and bile fistula animals that the same amount of radioactive label was incorporated into the neutral fats of the intestinal lumen, indicating that fat "digestion" was taking place. It is difficult to understand the nature of this absorptive defect in bile deficient animals especially in view of the proportionately greater lymphatic transport, but it would favor the concept that bile is not solely concerned with the digestive mechanism, per se.

One major criticism of Borgstrom's (13) work, which renders the interpretation somewhat difficult, is the short period of time between the establishment of a bile fistula and the thoracic duct fistula; especially, in light of the previous observation (60) that bile acids are present in the intestinal mucosa for a period greater than 24 hours following dietary diversion from the intestine. Certainly this does not invalidate the study, for a marked defect was noted in the biliary deficient animal despite the fact that bile acid deprivation may not have yet reached its maximum. These experiments support the previous investigations that in the absence of bile a significant quantity of fat is absorbed.

Borgstrom (13) also studied the relative specific

activities of the small intestinal phospholipids and neutral fatty acids. He observed that there was no significant difference between the normal and the bile deficient rats with respect to these specific activities. The relative specific activity of the free fatty acids was much higher in the bile fistula animals, which indicated to the author that dilution of the free labeled acids with non-labeled fatty acids liberated during lipolysis from carrier glycerides took place to a lesser degree in the bile fistula rats. This substantiates the view expressed by Annegers (2) that the action of bile extends beyond that of simple emulsification. This study also makes clear that bile deprivation does not preclude the intraluminal phase of intestinal fat assimilation. The fatty acids and glycerol are available for absorption in the bile deficient animals much as they are in the control animals.

Heersma et. al. (25) further published the observation of a higher fecal free fatty acid content in the bile deficient animal, with the magnitude of this fatty acid content approaching 100% of the excreted fat when a moderate amount of triglyceride fat such as lard was fed.

Wells et. al. (63) studied the absorption of fat utilizing I^{131} labeled triolein in control and bile fistula animals. He was unable to distinguish between the intact and the bile fistula dogs regarding fat absorption and alerts one to the possible insensitivity of his testing procedure.

Bernhardt et al (7,8,9) have studied the absorption of fats in rats and dogs maintained in a bile deficient state. They utilized fat balance studies with deuterium labeling of unsaturated fats, feeding the fats after saturating the animals body stores with the D isotope so that the deuterium atom would not migrate to other lipid or non-lipid recipient molecules and thus give erroneous results. However, a second criticism valid when this type of labeling procedure is carried out is that deuterium is heavier than hydrogen and as such the molecules containing deuterium may have a slower reaction rate than do the "normal" molecules, i.e. non-deuteriated.

The investigation of Bernhardt et al (7,8,9) supports the observations of Sperry (50), Shapiro et al (44), Coffey et al (17), Heersma and Annegers (25) and Pessoa et al (35), that the bile deficient animal is capable of absorbing a significant amount of lipid. However, these experiments of Bernhardt et al (7,8,9) are of particular interest in the authors interpretation of the mechanism of increased endogenous fecal fat excretion seen in the absence of bile acids. Bernhardt et al (8) interpret the increase to the loss of a blocking mechanism, operative in the presence of bile acids, which in the normal animal prevents excessive secretion of lipid by the intestinal mucosa and which in the absence of bile, where the blocking mechanism is lost, results in increased secretion of lipid in the feces.

Fecal Phosphorous

The chemical compounds including moieties of fatty acids; glycerol-phosphate and choline, serine or ethanolamine, have been commonly known as phospholipids. It should be recalled that any lipid containing phosphorous is a phospholipid by definition. It is only through constant usage that phospholipid has come to be considered synonymous by many investigators with the diglyceride-phosphate-nitrogenous base, usually calculated as lecithin.

These compounds have generated interest for many years. They first were suggested to be of prime interest in fat absorption by Miescher in 1897, as quoted by Verzar (60). At this time he postulated that they acted as an intermediate in fat absorption. This concept was supported by Bloor (12) who observed an increase in erythrocyte phospholipids during alimentary lipemia and he also thought the phospholipids of the blood represented an intermediate in fat absorption.

Sinclair (45) supports this concept with the evidence that as fats of high iodine number are ingested the mucosal lecithin content also has a high iodine number without any absolute increase in mucosal lecithin content. However, Eckstein (22), utilizing a canine thoracic duct preparation, was unable to record any constant increase in lecithin in lymph during fat absorption. This was later disputed by Sullmann and Wilbrandt in Verzar's laboratory as quoted by

Verzar (60), who demonstrated an increase in lymph phospholipid during fat absorption. The view point that phospholipid is an intermediate in fat assimilation has received verification in the form of experimental results which are consistent with the hypothesis, but which were not 'crucial.' At this time no one has devised a method which could approach the intestinal mucosa in a physiological absorptive state such that the flux of mucosal phospholipid could be accertained. Thus, biological significance of intestinal cell and lymph phospholipid remains without an adequate substantial explanation. This quandry is for the most part unavoidable in view of the technical difficulties in devising an approach to the problem which would yield unequivocal results.

In view of these differences and the conflicting bits of evidence, as well as the technical difficulties, it would seem that the crucial experiment will be difficult indeed to design. However, this does not necessitate abandonment of the problem for in view of practical usage the accumulation of information will permit one to examine the problem indirectly and through inference until the concept will be generally accepted.

In this light fecal elimination of organically bound phosphorous is of interest. Kim and Ivy (31) were the first authors to commit themselves in this area. They fed rats a basal "fat-free" diet with or without various types of fat added, and determined for each diet the fecal losses of total lipid and lipid phosphorous. They found that

the absolute amount of phosphorous recovered in the feces related to the type of fat fed. Thus, with the basal diet alone, fecal lipid phosphorous excretion was minimal and was not affected by adding either glycerol or cholesterol. However when triglyceride fat, such as corn oil, or fatty acid, as oleic acid, was added fecal lipid phosphorous excretion increased. The greatest increase occurred with the fatty acid. Addition of cholesterol alone, with the corn oil or oleic acid produced a further increase in fecal lipid phosphorous losses. In a similar manner they observed that in absolute milligrams of phosphorous corn oil fatty acids elicited a greater output of fecal lipid phosphorous than corn oil, itself. They also observed the elicitation of more fecal lipid phosphorous when unsaturated fatty acids, such as oleic acid, were fed than when saturated fatty acids, such as palmitic acid, were fed.

In synopsis, triglyceride fat, oleic acid, cholesterol, or cholesterol plus corn oil or oleic acid added to the basal ration increased the excretion of fecal lipid phosphorous. The unsaturated fatty acids and the fatty acids increased excretion to a greater degree than either the saturated fatty acids or the triglycerides, respectively.

Subsequent investigations of Pihl (35) inquiring into the interaction of free fatty acids and cholesterol during fat assimilation in rats also involved the measurement of fecal lipid phosphorous. He found that fecal lipid phosphorous increased from a range of 0.02 to 0.06 milligrams

per day, when either basal diet with or without cholesterol was added respectively, to a higher range of 0.24 to 3.42 mgm. phosphorous per day, when the free fatty acids were added with the cholesterol. With the feeding of unsaturated fatty acid, as oleic acid, the increase was greater than with the feeding of saturated fatty acid, as stearic acid. It is interesting that they could demonstrate no increase in fecal lipid phosphorous over the basal excretion of 0.02 mgm. per day when either cholesterol stearate or cholesterol oleate esters were fed.

From the above two studies, conducted in rats, it is apparent that fats stimulate the excretion of fecal lipid phosphorous to varying degrees. It is also evident that the physical and chemical characteristics of the fat are of importance; saturation, esterification, and chain length all apparently influence the magnitude of fecal lipid phosphorous excretion.

In contrast to the above two investigations in rats Pessoa, Kim, and Ivy (35) found that when normal dogs were fed corn oil or corn oil fatty acids there was no increase in fecal excretion of phospholipid over and above the basal fat free dietary excretion. However, when dogs were supplied with the same dietary fats after establishing a bile fistula, there appeared to be a significant increase in fecal lipid phosphorous in both corn oil and corn oil fatty acid feeding experiments as compared to the basal fat free diet. There did not appear to be a significant

difference between the corn oil triglyceride and free fatty acid feeding experiments in respect to the excretion of fecal lipid phosphorous.

It is noteworthy that in pancreatic duct ligated animals, treated in a similar manner, the increase in fecal lipid phosphorous was not observed, even in the face of a greatly increased fecal fat excretion. Further, they found quite a low fecal lipid phosphorous excretion in bile fistula dogs when they were fed saturated fat, as tallow. This suggests that unsaturated fats elicit more fecal lipid phosphorous than do the more saturated fats in the absence of bile acids. However, there is a paucity of data regarding this finding so as to make any conclusions extremely hazardous. Even so, the observation agrees with Kim and Ivy's (31) report of an increased fecal lipid phosphorous excretion when oleic acid and corn oil were fed as compared to the feeding of palmitic acid. On the other hand it is interesting to denote the difference in intact dogs as compared to intact rats. Dogs exhibit no difference in fecal lipid phosphorous excretion when fed triglycerides or free fatty acids, but rats excrete more fecal lipid phosphorous when they are fed free fatty acids than when they are fed triglycerides. Speculation concerning the significance of this point is futile due to the species difference. However, the reason for the increased elimination of lipid phosphorous in the feces

following the establishment of a biliary fistula remains to be explained. Also unexplained is the question of how individual, i.e. specific, fats and free fatty acids alter lipid phosphorous excretion in intact dogs.

Blomstrand (10) in 1955 investigated the absorption and transport of phospholipid in normal and bile fistula rats using thoracic duct fistulae and common bile duct cannulation procedures simultaneously. He found when feeding palmitic acid and/or oleic acid labeled with C^{14} in the carboxyl position and incorporated into phospholipid that the bile deficient animals absorbed less of the fed activity than the intact animals, and secondly that less of the absorbed activity is transported via the thoracic duct lymph.

Swell et al (58) investigated the physical characteristics of the fecal phosphorous-containing lipids. Previous authors above (31,35,36) assumed it to be a phospholipid and referred to it as such. This assumption appeared to be incorrect in view of preliminary chemical analyses done on lipid phosphorous obtained from the feces of rats. The first evidence was contained in the observation that this fecal lipid phosphorous had no choline moiety, and that after shaking with one normal hydrochloric acid no precipitate formed with the addition of methanol or acetone as you would expect if phospholipid, e.g. lecithin, cephalin, or sphingomyelin was present. Further investigations concerning the nature of this phosphorous containing

compound in terms of its solubility, salt-like nature (pK), and infra red spectral characteristics indicated to the authors that they were dealing with a calcium phosphate fatty acid complex rather than one of the compounds more commonly included in the term phospholipid, e.g. lecithin.

Swell et al (58) inquired into the effect of dietary fat on the fecal excretion of this calcium phosphate fatty acid complex in rats and found that triglycerides and saturated free fatty acids, such as palmitic acid, increased it only slightly over and above the excretion on a basal diet, but that unsaturated fatty acids, such as oleic acid, greatly increased the quantity excreted in the feces.

Studies on this calcium phosphate fatty acid complex were extended by Richards et al (39). They fed specific triglycerides and fatty acids to rats and found, as before, that oleic acid was a potent stimulant to the excretion of this complex. However, they found eicosenoic acid to be an even greater stimulant, while erucic acid was almost as potent as oleic acid, but it was a greater stimulant to the excretion of this complex than was palmitic, stearic, or nervonic acid. Trierucin was similar to free fatty acids in stimulating the output of this lipid phosphorous complex. Triolein and trilinolein gave no such increase, and in this regard resembled the saturated fatty acids. From the material extracted they were able to determine that the experimental results closely followed those calculated theoretically for the structure of a calcium

phosphate fatty acid complex regarding the ratio of calcium to phosphorous. The extracted material also had similar solubility properties to the synthetically prepared calcium phosphate fatty acid complex salts.

The biological significance of these observations has not been well delineated, but none the less one must be aware that these observations differ greatly from the original assumption that the fecal lipid phosphorous was a phospholipid closely resembling lecithin. The effect of this complex on the assimilation of certain fats has been raised by two groups of authors (22) and (57). As yet no further speculation about its significance is warranted. The one way in which one must change his thinking is about the excretion product. Now it would be erroneous to calculate the lipid phosphorous excreted in the feces assuming it to be predominately lecithin.

In this regard Carroll and Richards (15) calculated the amount of calcium phosphate fatty acid complex on the basis of fecal lipid phosphorous determinations after feeding various fats to rats. They found that rats fed free fatty acids of olein and linolein contained more phosphorous in their feces than did rats fed triolein or trilinolein. It should be emphasized that the rats receiving the fatty acids also had more lipid content in their feces than did the rats fed the triglycerides. Those fed tristearin excreted more phosphorous in their feces than those fed stearic acid. Those fed palmitic acid or tripalmitin

showed no phosphorous contained in their feces. It should be noted that a considerable amount of fecal fat was present when tripalmitin was fed, but still no fecal phosphorous was present in measurable quantities.

One qualification which must be made in reference to these studies which identified the "phospholipid" as calcium phosphate fatty acid complexes, is that all of the work which has been done to date was done on rat feces. Although this does not detract from the investigations, it precludes any conclusions regarding the nature of fecal lipid phosphorous compounds in other experimental animal species; e.g. the canine.

In summary: Very little is known about fecal lipid phosphorous, the effects of dietary fats or of bile upon its excretion. There is some evidence that in rats the fecal lipid phosphorous is not the classically conceived phospholipid, lecithin, but is a calcium phosphate fatty acid complex and that the quantitative elimination of this complex depends to some degree upon the type of fat ingested, i.e. its saturation, esterification and chain length.

STATEMENT OF THE PROBLEM

The problem for which the preceeding Review of the Literature is the background can perhaps best be discussed in three parts. The first part of the problem deals with endogenous fecal lipid excretion in normal and bile fistula dogs with essentially no fat in the diet. The second part deals with the fecal losses of lipid following the ingestion of specific triglycerides and the sodium salts of the respective fatty acids in normal and biliary fistula dogs. The third part deals with fecal lipid phosphorous excretion in these same dogs.

The first consideration, that of endogenous fecal lipid excretion, is of importance in any investigation dealing with the absorption of fats from the intestine when the method of choice is the fat balance study.

In this investigation, as in any other investigation utilizing the fat balance technique, it is necessary to measure endogenous lipid excretion in order to apply as a correction factor for fat feeding studies. This would assure one that the change, if one occurs, is in the area designated for investigation and not due to an uncontrolled variation in endogenous lipid excretion.

In studies in which comparisons are drawn between normal and bile fistula dogs the endogenous fecal lipid losses take on added importance because it has been shown that in the absence of bile there is not only a marked

elevation in the absolute endogenous fecal loss (30,34), but also an apparent selective excretion of saturated components of these endogenous compounds (50). This suggests that bile either selectively facilitates absorption of the saturated components of the endogenous lipids or reduces the rate at which these saturated compounds are secreted into the intestinal lumen. Information of this type has been gained by following the change in the iodine number of the fecal lipids.

Also under consideration is the fecal lipid losses in normal and bile deficient animals when the sodium salt of the respective fatty acid is added to the basal diet. This maneuver assumes more importance when one considers the lipolytic theory of fat absorption advocated by Verzar (60). This theory presents bile as an important agent in the digestion of fats which acts by "solubilizing" the fats so that enzymatic lipase may break the fats down into smaller molecules. The ultimate product of this digestion would be the free form of fatty acid, and it has been contended that it is in this state that much of the fat absorption takes place. If this is the true schema of fat assimilation it appears reasonable that in the absence of bile, whose role is supposedly only that of solubilizing the fats for digestion, assimilation of a sodium salt of a fatty acid should be as readily absorbed as it is in the intact animal.

The present investigations were undertaken with the knowledge that the studies with which one is confronted in the literature have been carried out using mixtures of fat in the diet. Only in one or two instances have more specific fats been used. Thus, the second consideration is the factor of absorption of specific fats by the intestine in normal and bile fistula dogs. It is apparent from the very early literature that in the normal animal tristearin is poorly absorbed by the intestine while triolein is extremely well absorbed (41). However, in view of the equal chain length of these two fats, it is essential to compare their absorption from the intestine, especially when degree of saturation is a prime consideration. Otherwise, the variable of chain length would also have to be considered. These two fats are widely found in natural food sources of man and animals so that the problem of the physiological significance of studies involving the introduction of a foreign lipid-like substance is avoided.

In the literature review it was pointed out that little information is available regarding phospholipids when various lipids were fed to rats or dogs.

The fecal lipid phosphorous assumes more significance as one considers the increased excretion of these compounds in biliary deficient dogs. The normal dog excretes only trace amounts of phosphorous which increases to significant measurable levels in the biliary deficient animal (35). Further, there is the suggestion that the feeding of

unsaturated fatty acid is associated with a significantly higher fecal lipid phosphorous loss than is the feeding of equivalent amounts of saturated fatty acids in the normal animal (31,35).

When studying the fecal lipid phosphorous excretion, one becomes aware of the fact that very little information is available reagarding the effect of specific dietary fats of equal chain length, but of differing saturation, in either normal or bile fistula dogs.

Another facet of fecal lipid phosphorous excretion is its endogenous loss in the normal and bile fistula state. In the fed animal this bears on the question of interpretation of results in a manner similar to that described for total lipid.

It is the purpose of this investigation, therefore, to elucidate the nature of fecal fat excretion in the normal and bile deficient dog. Special emphasis is placed on the effect which the addition of specific fats to the diet have on the fecal fat excretion, its degree of saturation, and the lipid phosphorous excretion. The fats selected were two triglycerides and the sodium salts of their respective fatty acids of equal chain length, but of differing degrees of saturation. These were so selected to demonstrate differences in the parameters of fecal fat excretion discussed above.

MATERIALS AND METHODS

Experimental Animals:

Dogs weighing between 9.5 and 13.8 Kg. were chosen as the experimental animals to be used throughout this investigation.

Diet:

The animals were fed a synthetic "fat-free" basal ration to which the test fats were added. The synthetic basal diet had the following composition:

Casein	24%
Cellulose	14%
Corn Starch	16%
Sucrose	40%
Salt Mixture, U.S.P. XIV	4%
Vitamin Fortification Mixture	2%

This synthetic diet, containing approximately 3.2 Calories per gram, was mixed with water to a semi-liquid consistency and fed at the rate of 80 Calories per Kg. body weight per day.

Test Fats:

The test fats used included triolein (obtained as commercial olive oil), tristearin (as technical grade 97% Mathison, Coleman and Bell) and the sodium salts of the respective fatty acids of these two triglycerides. The sodium salts were synthesized from technical grades of the free fatty acids by dissolving the fatty acids in hot alcohol

and saponifying with sodium hydoxide (69). After drying the soaps were ground to a coarse powder with a hand grinder.

Feeding:

Each of the test fats was originally planned to be fed to the animals at the rate of 5 gm./Kg./D added to the basal diet. However, due to individual eating habits, varying amounts of basal diet containing the test fats remained uneaten at the end of the 24 hour period allowed for consumption. Therefore, the amount of diet plus test fat ingested varied considerably from day to day. Occasionally the animals refused to eat any portion of a diet containing 5 gm./Kg./Day of the test fat. This was especially true of sodium oleate due to the unpalatable taste which could not be disguised by the basal diet. In such an event the animals were fed the test fat at the rate of 2.5 gm./Kg./Day in the hopes that they would eat it at this reduced level.

The basal diet, which tends to separate on standing, was thoroughly mixed before feeding. If a test fat was to be fed it was added just prior to feeding. The triolein and tristearin were mixed thoroughly into the basal ration in a small one quart waring blender. This could not be accomplished with the soaps which formed a thick pasty diet and made manual mixing necessary. The daily test diet was left in the animals cage for a 24 hour period. At the end of this time if any portion of the animals diet remained uneaten the amount was weighed and recorded before it was discarded.

An experiment period consisted of a seven day feeding schedule during which time the animals received the same diet, either basal or fat containing, at regular 24 hour intervals.

Fecal Collection:

The animals were housed in metabolic cages, the floor of which sloped to a center opening through which the urine was made to exit. These metabolic cages facilitated the quantitative collections of fecal material. The collection period consisted of the last five 24 hour periods of the seven day feeding schedule. At the end of each 24 hour period the feces were quantitatively collected and stored under a 0.2% sulfuric acid solution in the refrigerator to retard bacterial action. Following collection, the five day pooled specimen was homogenized in a tared Waring Blender of one gallon capacity, Model CB-3. The homogenate was weighed to the nearest gram and a portion was stored in the refrigerator until it was analyzed. To any one dog only two test fats were fed; the triglyceride and respective sodium soap. Before any of the test fats were added to the diet a feeding period of basal diet alone was carried out to establish "endogenous" fecal losses.

Preparation of Biliary Fistula:

The biliary fistula was constructed as described by Kapsinow, Engel and Harvey (15). This technique, commonly referred to as the internal biliary fistula, diverts bile from the intestine through a conduit provided by the pelvis

of the right kidney. The procedure is essentially as follows: Under nembutol anesthesia and using aseptic techniques, the the common bile duct is divided between double ligatures, an opening is made through the wall of the drained gall bladder and the gall bladder is anastomosed into the pelvis of the longitudinally bisected right kidney. The bile is thus prevented from entering the intestine and is eliminated in the urine.

To assure complete absence of bile the metabolic cages promptly vented the voided urine from the cage and obviated the possibility of voluntary ingestion of the bile containing urine. In all biliary fistula dogs fecal urobilinogen was determined to test for the presence of bile in the intestine (62)(65).

Each of the animals was allowed to recover at least three weeks and appeared to be in good health without evidence of jaundice in every case. Following the recovery period the basal diet and test fat diets were again fed during an experimental period and fat balance studies carried out exactly as they were described before the bile fistula. In each case the same types of fat were fed before as after bile diversion.

In the above manner a total of 69 experiments were performed on 11 animals.

Total Lipid Extraction Method:

The method for measurement of the lipid substances in the collected fecal material was a gross modification of

the 2:1 chloroform : methanol ratio method of lipid extraction developed for use in blood lipid measurements by Sperry and Brand (62). Since the method is new in its present application it will be described in some detail below.

This method uses a chloroform : methanol mixture which has a final dilution ratio that is very close to 1:1. The fecal aliquots, approximately 5 ml., were obtained from the five day pooled specimens. The aliquots were weighed in tared 50 ml. beakers and washed with absolute methanol into the 100 ml. glass stoppered mixing cylinders containing 1 ml. of concentrated HCl. This mixture was diluted to 50 ml. with additional methanol and a small amount of chloroform (less than 10 ml.) was added.

The unstoppered cylinders were then immersed in a boiling water bath until the mixture was just seen to boil. The cylinders were then removed from the water bath and additional chloroform added, so that the total volume approached 100 ml. The glass stoppers were placed in the cylinders and they were left to cool to room temperature. When they had reached room temperature the final dilution to 100 ml. was made with additional chloroform.

At this time the cylinders were shaken briefly and immediately filtered through Whatman number 2 filter paper. Aliquots of 30 ml. were taken from the filtrate and transferred forcibly, with a pipettor into 50 ml. centrifuge tubes containing 10 ml. of distilled water, forming an emulsion.

The emulsion was centrifuged at 1500 R.P.M. for ten minutes to facilitate separation of the aqueous and chloroform layers. At the end of this time sharp delineation into an aqueous supernatant and chloroform subnatant fraction was effected. A metal tube 3 mm. in diameter was inserted into the bottom of the centrifuge tube through a cork stopper so that positive pressure could be applied with a hand bulb to transfer the subnatant into tared 25 ml. Erlenmeyer flasks.

The volatile solvents and the water contained in the chloroform solution were evaporated from the tared flasks under a stream of nitrogen on a slowly boiling steam bath. When the tared flasks containing the extracted lipid were removed from the steam bath they were immediately placed in a glass dessicator which was promptly evacuated with a water suction pump and the atmosphere replaced with nitrogen. This was repeated twice after all flasks had been placed in the dessicator and it was left containing a slight negative pressure. When the flasks were cool they were again weighed and the amount of lipid in the extraction aliquot was obtained by subtraction. The amount of lipid, as chloroform soluble substances, present in the fecal aliquot was obtained by multiplying the above extraction aliquot by 3.333.

The above described method for measuring fecal total lipid, as chloroform soluble substances, was evaluated by recovering known amounts of triolein, tristearin, sodium oleate and sodium stearate from the extraction system alone

and from fecal suspensions to which these lipids had been previously added. They were added in known amounts and extracted as described above to include the range of values one might expect to recover in the feces of normal and bile fistula dogs. The recoveries of the amounts which were added to feces were plotted against the amounts recovered from feces. The recovery curves are shown in Figures 1 and 2.

The triolein and tristearin regression equations had regression coefficients which did not differ statistically from B = 1, indicating that the amount extracted was a true measure of the amount in the feces.

The regression coefficients of sodium stearate and sodium oleate on the other hand both differed significantly from B = 1 ($P<0.001$), suggesting that recoveries were incomplete. However, the fact that the relationship between the amounts added and the amounts recovered was linear throughout the entire range of values means that the percentage error was constant throughout. This constant percentage error could be explained on the basis of having added an impure compound to the fecal suspensions rather than on the basis of an error in extraction. That this is likely is suggested by the fact that the fatty acids utilized for soap synthesis were technical grade products with the purity unspecified.

The sensitivities of the extraction of these four lipids were determined from the fiducial limits about the

FIGURE 1 Total lipid extraction method:

Relationship between known amounts of triolein and tristearin added to feces and the recovery of each using the chloroform:methanol extraction system. The regression line is presented with the regression coefficient and its standard error.

FIGURE 1

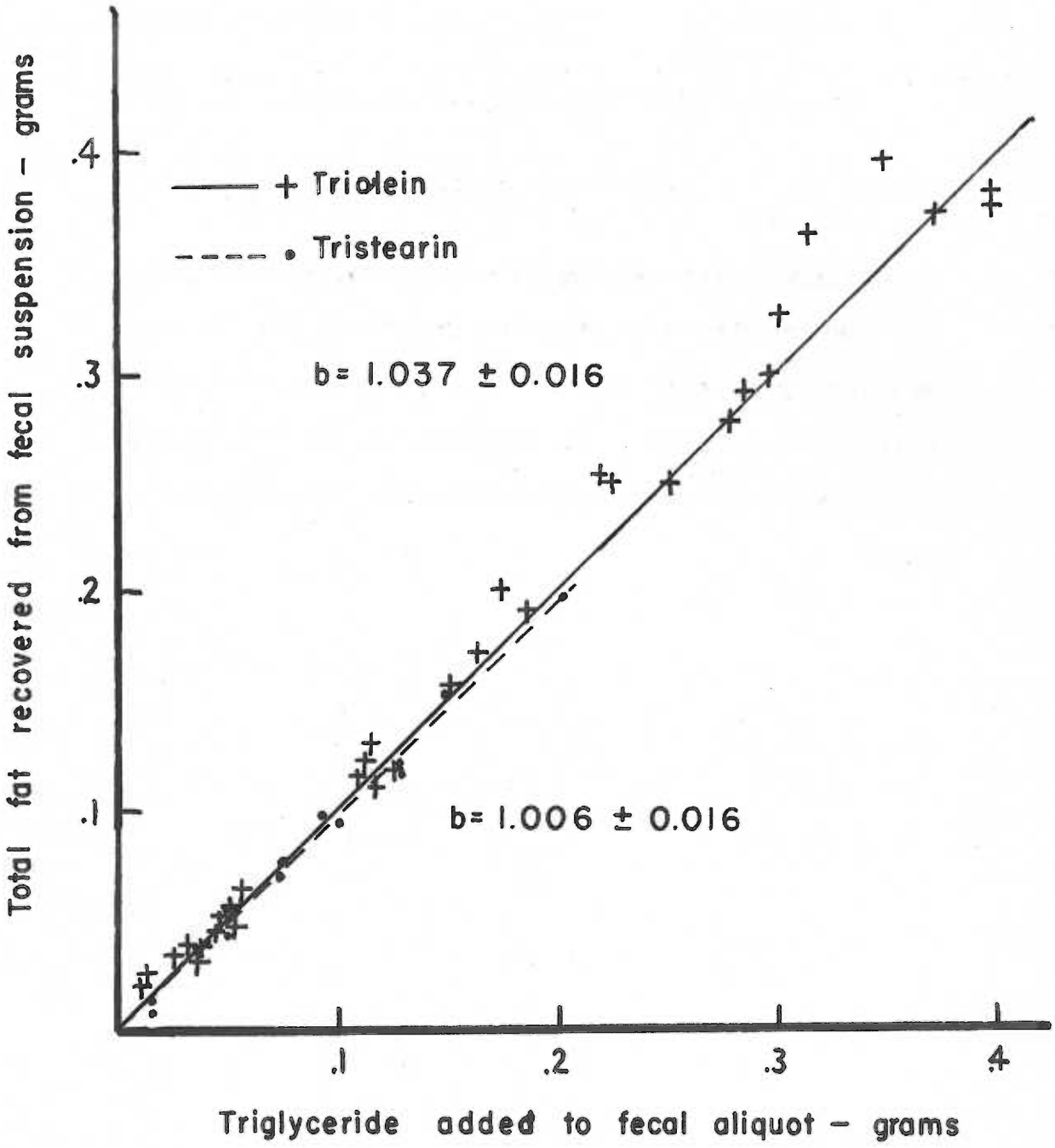
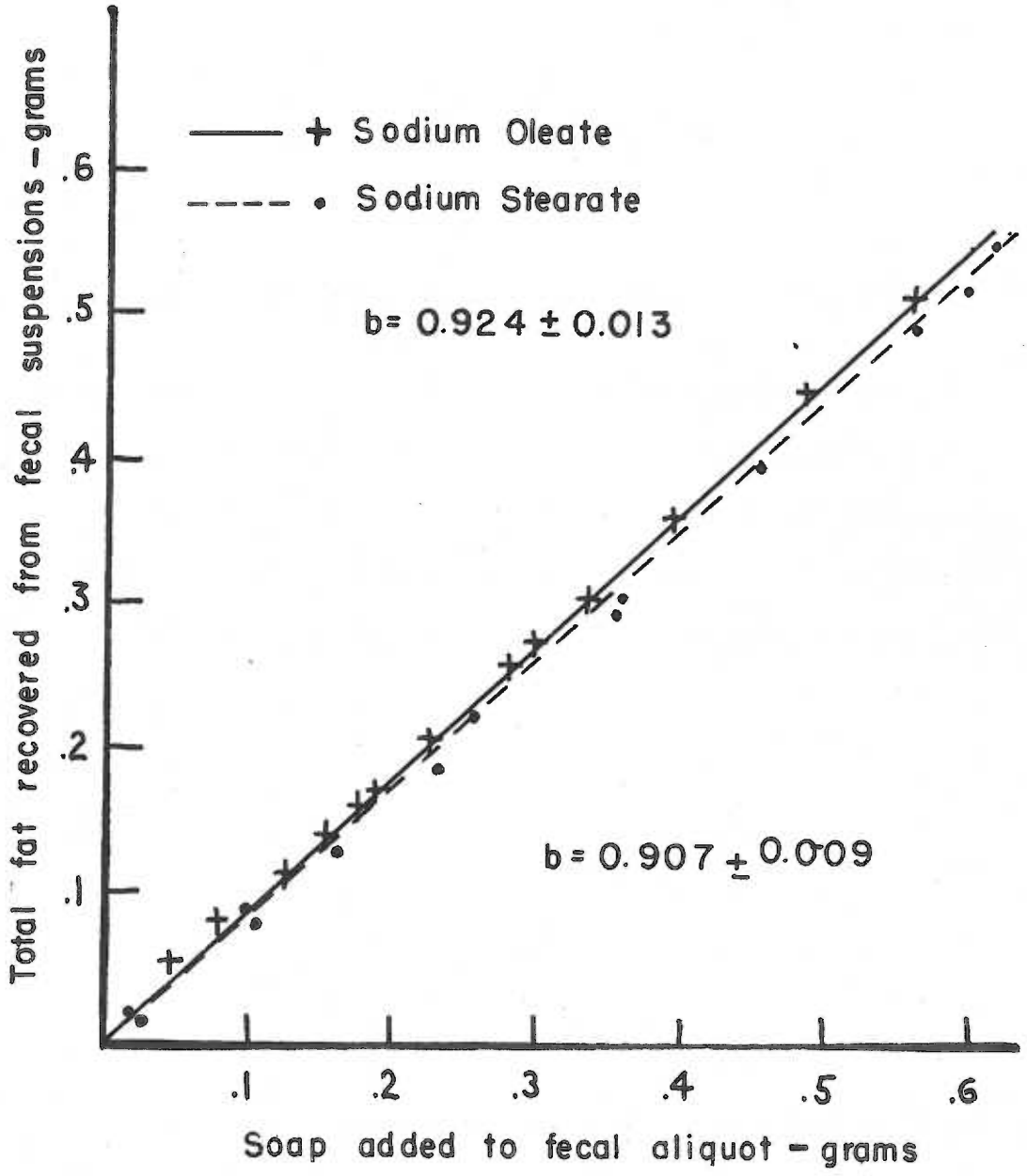

Total fat recovered from fecal suspension – grams
.4
.3
.2
.1
+ Triolein
• Tristearin
b= 1.037 ± 0.016
b= 1.006 ± 0.016
.1
.2
.3
.4
Triglyceride added to fecal aliquot – grams

FIGURE 2 Total lipid extraction method:

Relationship between known amounts of sodium oleate and sodium stearate added to feces and the recoveries of each using the chloroform:methanol extraction system. The regression line is present with the regression coefficient and its standard error.

FIGURE 2


+ Sodium Oleate
• Sodium Stearate
b= 0.924 ± 0.013
b = 0.907 ± 0.009
Total fat recovered from fecal suspensions - grams
.1
.2
.3
.4
.5
.6
Soap added to fecal aliquot - grams

mean extraction value of fats added to feces. These sensitivities are shown below (at $P < 0.05$) for each of the lipid studies:

Triolein	44.0 mgm.	equivalent - 0.0008	gm./Kg./D
Sodium Oleate	15.6 mgm.	equivalent - 0.0002	gm./Kg./D
Tristearin	8.4 mgm.	equivalent - 0.0003	gm./Kg./D
Sodium Stearate	15.2 mgm.	equivalent - 0.0003	gm./Kg./D

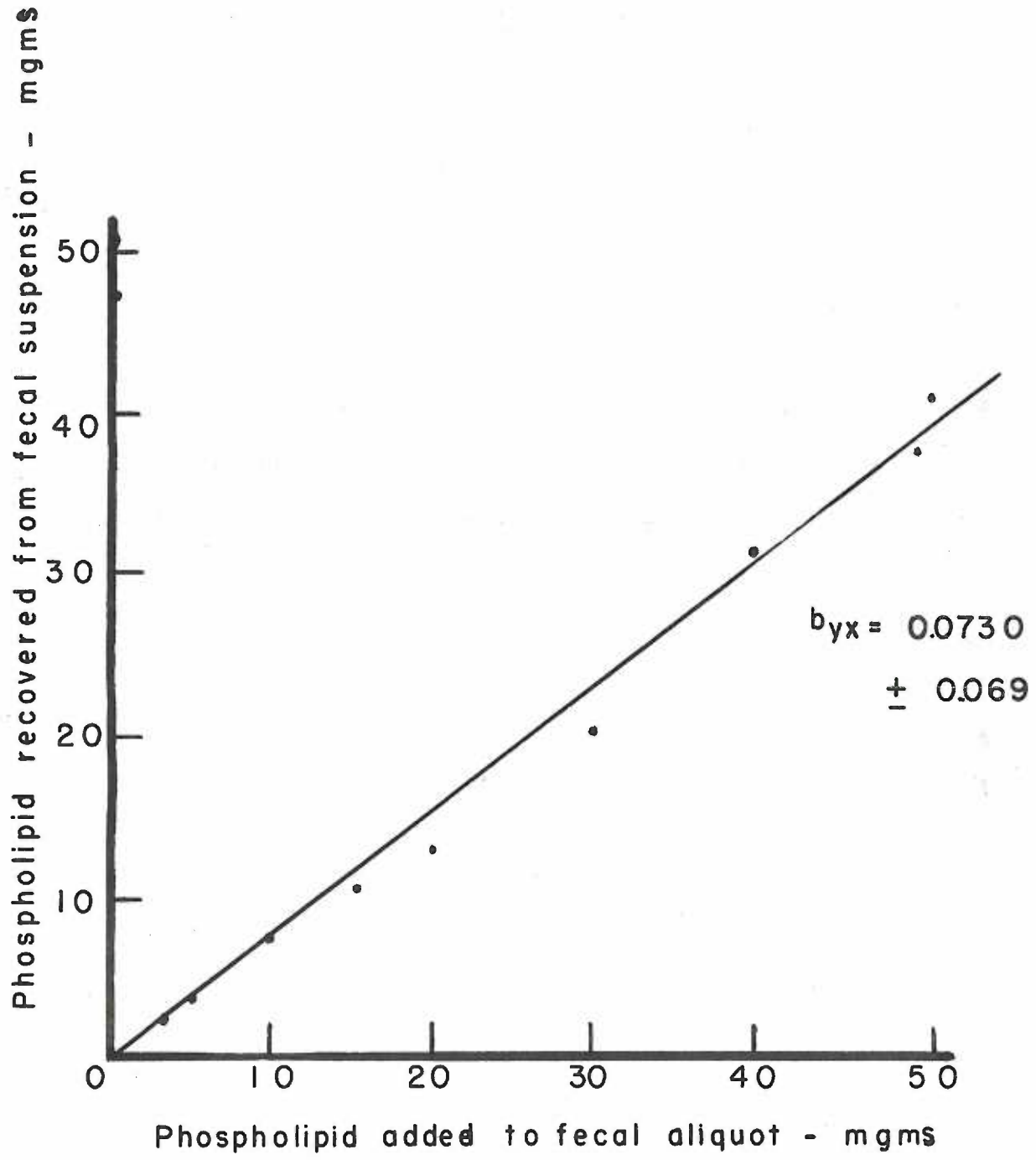
The fecal lipid phosphorous determination method was tested using egg lecithin. This compound was added to various fecal suspensions, extracted as per total lipid, and the phosphorous determined on the total lipid extract. The amounts recovered were plotted against the amounts added, expressed as lecithin (lipid phosphorous X 24.1). The results are shown in Fig. 3.

The regression coefficient differs significantly from $B = 1$ ($P < 0.01$). In this regard the consideration of the purity of the product used for testing was questioned, since a statement concerning its purity was not available. However, the possibility of an error in the determination must also be considered. Of the amount of egg lecithin added to fecal suspensions 94% to 96% was recovered as total lipid by chloroform methanol extraction. Only 73% of this was recovered as lecithin by the phosphorous method. This means either that the added material was not pure lecithin or that there was a constant loss of phosphorous as determined by the

FIGURE 3 Total lipid extraction method:

The relationship between known amounts of egg lecithin added to feces and the recovery using the chloroform:methanol extraction system. The regression line is presented with the regression coefficient and its standard error.

FIGURE 3


Phospholipid recovered from fecal suspension - mgms
50
40
30
20
10
0
10
20
30
40
50
Phospholipid added to fecal aliquot - mgms
b_{yx} = 0.0730
± 0.069

Fiske and Subbarow method (24). In either event, the relationship between added and recovered amounts is linear over the entire range of values.

The sensitivity of this method was determined from the fiducial limits about the mean recovery of phospholipid added to fecal suspensions. The smallest difference this method is capable of detecting at ($P < 0.05$) would be 7.19 milligram lecithin, or approximately 0.006 mgm. of fecal lipid phosphorous per Kg. body weight per day.

Iodine Number: The degree of saturation of the recovered chloroform soluble substances was accomplished with Yashuda's modification of the Rosemund Kuhhenn Iodine number determination (63). This was accomplished using elemental bromine as the reacting substance catalyzed by pyridine sulfate. The reaction takes place in the dark for a 15 minute period. At the end of this time the reaction is stopped by the addition of 0.5 ml. 10% KI and titrated with 0.02 Normal sodium thiosulfate. The sodium thiosulfate prepared was standardized against potassium bi-iodate. Soluble starch was used as the indicator substance.

RESULTS

The results obtained might best be described under the headings of the variables measured, i.e. fecal total lipid, fecal lipid iodine number, and fecal lipid phosphorous excretion. In each of these categories five diets were fed to normal and bile fistula animals under as nearly identical circumstances as possible. The raw data for the basal diet alone, triolein, tristearin, sodium oleate and sodium stearate added to the basal diet are presented in the appendix (Tables III to VIII inc.).

Total lipid: The basal diet fed to normal and bile fistula dogs contained approximately the same amount of fat in both groups, ranging from 0.099 to 0.129 grams/Kg. body wt./Day. The normal animals excreted a mean of 0.126 gm./Kg./D of fat compared to a mean excretion of 0.203 gm./Kg./D of fat in these same animals following bile deprivation. These mean values were found to differ significantly at $P < 0.02$.

When triolein was added to the basal diet the fecal fat excretion increased markedly in the bile fistula animals, to a mean value of 1.679 gm./Kg./D. In the normal animals fed triolein very little increase was noted. They averaged only 0.152 gm./Kg./D fecal fat excretion. The paired comparison analysis of the data demonstrated that even with a lower fat intake in the bile fistula animals, average = 2.8 gm./Kg./D compared to 4.3 gm./Kg./D in normal animals,

each experimental pair exhibited a significantly greater amount of fecal fat loss, $P < 0.01$.

Since the animals ate varying amounts of the diet, the range of intakes with triolein feeding were such as to permit an expression of fecal fat loss as a function of intake for both groups. (Fig. 3) The normal and bile deficient groups are then compared by linear regression analysis. (See Table 2). The data upon which the regression analysis was run in all cases included that obtained in the animals fed the basal diet alone, in order to incorporate the information concerning endogenous fecal fat losses. The regression coefficient of 0.526 in the bile deficient state differs significantly from 1 and indicates that approximately 48% of the fed triolein was absorbed in the absence of bile. In contrast to this the regression coefficient in the normal animals was only 0.057 indicating that approximately 95% of the fed triolein was absorbed in that group.

The experiments in which sodium oleate was fed to normal and bile deficient animals demonstrated that this soap was well absorbed in the normal animal. The regression coefficient was found to be 0.073 and appears comparable to the experiments in normal animals fed triolein. On the other hand, when this fat was fed to bile deficient animals, the fecal fat loss averaged approximately 42% of the fed amount as indicated by the regression coefficient of 0.4260. In this respect the results parallel somewhat the defect in absorption seen in triolein feeding. In the cases of both

TABLE 1

AVERAGE FECAL FAT EXCRETION

Normal or Bile Fistula	Number of Dogs	Mean Wt. Kg.	Added Fat Ingested Mean gm./Kg./D	Fecal Fat Loss Mean gm./Kg./D	"P" Value *
N	11	11.4	0	0.126	
BF	11	11.4	0	0.203	0.02
			Triolein		
N	6	11.7	4.3	0.152	
BF	6	11.7	2.8	1.679	0.01
			Sodium Oleate		
N	6	11.7	3.3	0.357	
BF	6	11.7	2.0	1.151	0.01
			Tristearin		
N	5	10.8	5.0	3.651	
BF	5	10.7	4.4	4.928	0.15
			Sodium Stearate		
N	5	10.8	4.2	3.419	
BF	5	10.8	3.9	3.538	0.08

* "P" Value 0.05 is regarded as significant. This value is a statement of the probability that the mean differences of the paired comparisons in normal and bile fistula animals could result from random sampling by chance.

fats there were significant amounts absorbed in the bile deficient state. However, comparison of the regression coefficients of the bile deficient animals fed triolein and sodium oleate demonstrates statistically significant difference at $P < 0.005$, indicating that the bile deficient animal absorbs sodium oleate more efficiently than triolein.

Sodium stearate added to the basal diet was shown to be very poorly absorbed both in the normal and bile deficient state. The regression coefficient in the normal state was found to be 0.799 indicating approximately 20% absorption compared to a more than 90% absorption in the case of both triolein and sodium oleate. Comparing fecal fat losses in the normal and the bile deficient state when sodium stearate was fed, there was indication of a significant increase in fecal fat loss after bile deprivation. The regression coefficient in the bile deficient state was found to be 0.871 indicating approximately 13% absorption. The magnitude of the absorptive defect in the bile deficient state with sodium stearate feeding would then be in the order of 7% compared to 47% in the case of triolein and 37% in the case of sodium oleate.

Tristearin added to the basal diet of normal and bile fistula animals was also found to be very poorly absorbed. Since the range of intake was small, regression analysis was not possible with this lipid. However, no significant differences, by paired comparison analyses, could be detected between the normal and bile deficient state. The amount

TABLE 2

REGRESSION ANALYSIS - FECAL FAT EXCRETION

		b ± Standard Error	a	Correlation Coefficient	"P" Value
Triolein	Normal	0.057 ± 0.010	0.004	0.13	
	Bile Fistula	0.526 ± 0.030	0.21	0.9965	0.001
Sodium Oleate	Normal	0.073 ± 0.002	0.129	0.7946	
	Bile Fistula	0.426 ± 0.002	0.199	0.9806	0.001
Sodium Stearate	Normal	0.799 ± 0.027	0.040	0.9855	
	Bile Fistula	0.871 ± 0.016	0.210	0.9993	0.05

All regression coefficients are statistically different than zero, P ' 0.01

absorbed in both groups was so small as to be negligible.

Iodine number: The iodine numbers of the total fecal lipids extracted from the feces are summarized in Table 3. It is evident that the fecal fat excreted in the bile deficient state does not differ from that excreted in the normal state in terms of its iodine number, for all experiments. The only difference noted was that which could be attributed to the type of fat which was fed. As expected, since the saturated varieties were poorly absorbed, the fecal lipid iodine number was low in those instances where saturated lipids were fed. In the case of unsaturated lipid feeding the fecal lipid iodine number could not be shown to differ from that in which no fat was added to the diet.

Fecal lipid Phosphorous: The values for these determinations in the total lipid extracted from the feces of normal and bile fistula animals are also summarized in Table 3. These figures, as with the iodine number data, demonstrate no significant difference between the normal and bile deficient state except in the case of tristearin feeding. When tristearin was added to the basal there was a significant increase in the fecal lipid phosphorous in the bile fistula dog compared to normal lipid phosphorous excreted, at $P<0.001$.

TABLE 3

IODINE NUMBER AND FECAL LIPID PHOSPHOROUS

Type Fat Added to Basal Diet	Iodine Number Mean		P* Value	Fecal Lipid Phosphorous gm./Kg./D		P* Value
	Normal	Bile Fistula		Normal	Bile Fistula	
None	40.4 ± 10.8	37.7 ± 11.4	0.40	0.40 ± 0.16	1.10 ± 0.66	0.25
Triolein	49.7 ± 14.4	36.8 ± 10.5	0.25	0.38 ± 0.14	0.46 ± 0.06	0.50
Sodium Oleate	48.2 ± 13.7	64.3 ± 25.1	0.20	0.41 ± 0.21	0.49 ± 0.18	0.70
Tristearin	4.3 ± 4.8	4.8 ± 2.4	0.30	0.29 ± 0.05	0.47 ± 0.02	0.001
Sodium Stearate	2.3 ± 0.63	3.5 ± 0.62	0.30	0.37 ± 0.18	0.50 ± 0.27	0.40

P* Value 0.05 is considered significant. This value is a statement of probability that the differences between normal and bile fistula animals could result from random sampling by chance.

FIGURE 4 Total Fecal Lipid - Triolein Feeding

The relationship between the amount of triolein added to the diet and the amount of total lipid recovered in the feces, in normal and bile fistula dogs. The regression line is presented with the regression coefficient and its standard error.

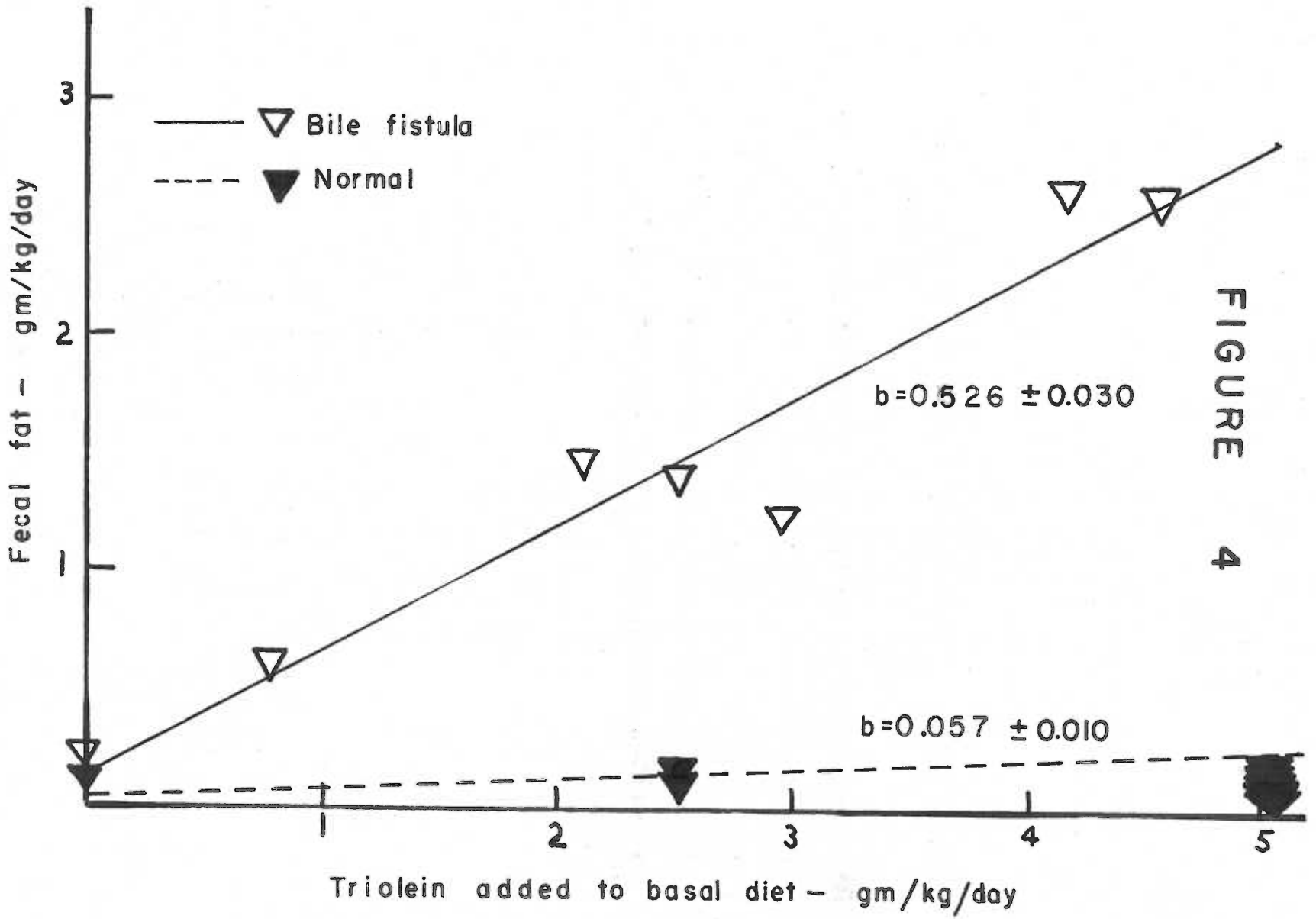

Bile fistula
Normal
Fecal fat – gm/kg/day
3
2
1
b=0.526 ±0.030
b=0.057 ±0.010
1
2
3
4
5
Triolein added to basal diet – gm/kg/day

FIGURE 4

FIGURE 5 Total Fecal Lipid - Sodium Oleate Feeding

The relationship between the amount of sodium oleate added to the diet and the amount of total lipid recovered in the feces, in normal and bile fistula dogs. The regression line is presented with the regression coefficient and its standard error.

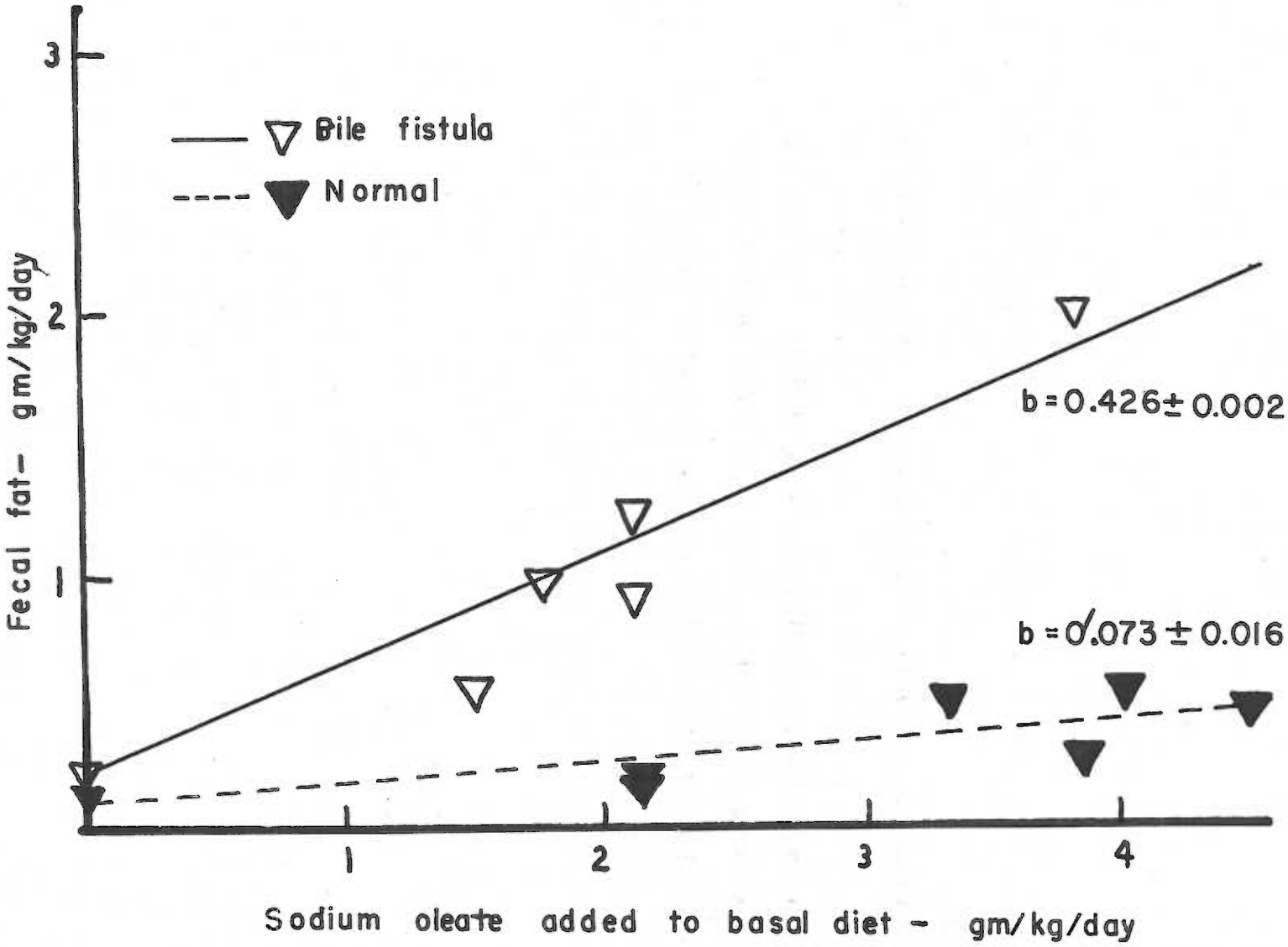
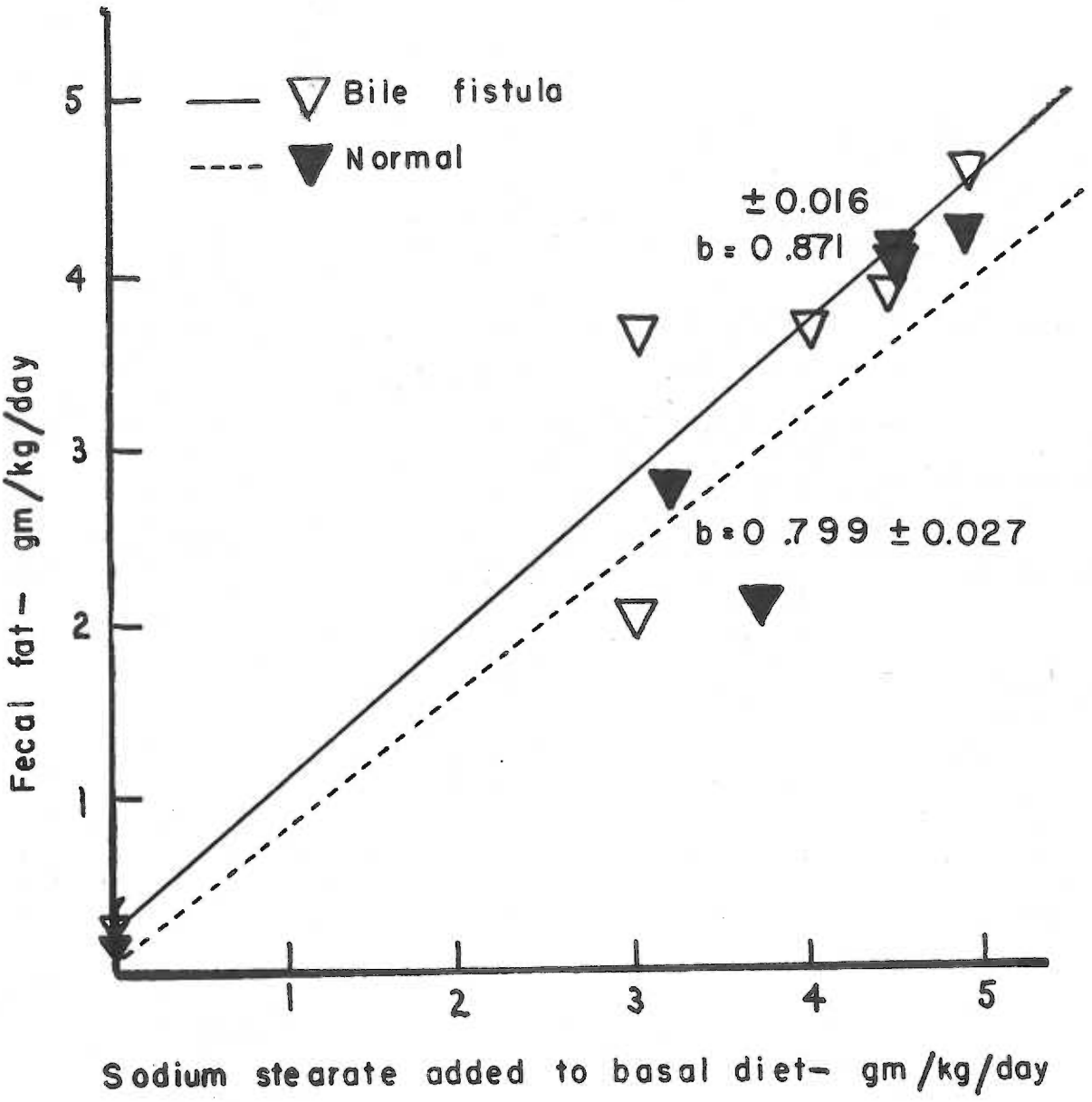

Bile fistula
Normal
Fecal fat - gm/kg/day
3
2
1
b = 0.426 ± 0.002
b = 0.073 ± 0.016
1
2
3
4
Sodium oleate added to basal diet - gm/kg/day

FIGURE 5

FIGURE 6 Total Fecal Lipid - Sodium Stearate Feeding

The relationship between the amount of sodium stearate added to the diet and the amount of total lipid recovered in the feces, in normal and bile fistula dogs. The regression line is presented with the regression coefficient and its standard error.

FIGURE 6


Bile fistula
Normal
±0.016
b = 0.871
b = 0.799 ± 0.027
Fecal fat – gm/kg/day
Sodium stearate added to basal diet – gm/kg/day
1
2
3
4
5

DISCUSSION

It was shown in the results that the animals fed the basal diet alone exhibited a significant increase in fecal fat excretion when they were deprived of bile. This finding is consistent with the observations of Sperry (50,53), Annegers et al (25) and Pessoa et al (35), but does not agree with the study of Searle and Annegers who reported no difference in the quantity of endogenous fecal fat excreted in the normal compared to the bile fistula animal (43). In the present series there seems to be little doubt that the increase of endogenous fecal fat observed in the bile deficient animal represents a real and significant change. This apparently increased endogenous fecal fat excretion in bile deficient animals and has been speculated upon for many years in terms of its origin and its biological significance. In terms of its origin, Rony et al (40) described a diminished transport in the quantity of thoracic duct lymph lipid following a sub-total enterectomy in dogs.

Bloor, (12) suggested that this endogenous lipid arose directly from the lipids of the blood stream by secretion or diffusion, while others (21,40) have suggested that they represented a product of the mucosal cells which was actively secreted into the lumen. The most recent suggestion comes from Bernhardt et al (7,8,9). These authors believe that the bile acids act in the intestinal mucosa in some manner to inhibit the secretion of fats into the intestine.

In this respect when a bile fistula is established the inhibition is removed and fecal fat is excreted in increased amounts. This differs somewhat from the concept which has existed for many years previously. It states that the bile salts act in the intestine to facilitate the absorption of the intestinal lipids which are constantly being introduced into the intestine by whatever manner. Both concepts are consistent with the observations showing the presence of lipid substances in the resting secretions from Thiry-Vella loops (1,21) as well as in fasting ileostomy secretions (56). However, the measurements utilized in the investigation presented here do not offer any further information concerning this facet of fat absorption and further speculation is not warranted.

Sperry (50,53) presented data showing a decrease in the solid to liquid fatty acid ratio in the feces of bile fistula compared to normal animals, suggesting that bile acids might facilitate the absorption of saturated fats to a greater degree than unsaturated fats. In the present study the question was persued by relating the iodine number of the fecal lipids and the quantitative fecal lipid losses to the type of fat which was fed in the normal and the bile deficient states. The data presented in the results section showed that the bile fistula animal has a greater defect in absorption of the long chain unsaturated triglyceride than it had in either the corresponding unsaturated or saturated soaps. The absorption of the saturated fats in the normal

and bile fistula animals was extremely low, in fact the tristearin appeared to be absorbed in only negligible quantities, whether or not bile was present in the intestine. On the other hand, the sodium stearate was absorbed to a significant, although small degree by both normal and bile deficient animals. The greater defect in unsaturated compared to saturated fat absorption would indicate that the bile acids were facilitating the absorption of the unsaturated fats to a greater degree than the saturated ones.

Additional support for this observation was obtained from the iodine number determinations. With the feeding of unsaturated fat the iodine number of the fecal lipids did not differ from that obtained in experiments in which the basal diet alone was fed. Also, following bile fistula there was no demonstrable change in the iodine number of the fecal fats regardless of the type of fat fed. This would suggest that bile, per se, did not seem to influence the iodine number of the fecal lipids. If bile acids were to facilitate the absorption of the saturated fats to a greater degree than the unsaturated fats, a decrease in the iodine number would have been expected following the removal of bile. This did not occur when the animals were fed either the basal diet alone or with unsaturated fat additions. There was a marked decrease in the iodine number of the saturated fat feeding experiments which was probably the result of very poor absorption of these fats and a resulting large fecal residue of dietary fat.

The greater defect in the absorption of triglycerides in the bile deficient state would indicate a more efficient absorption of the sodium salt of the respective fatty acids. This finding is consistent with the old idea of Verzar (60), who concluded that fatty acids were the end products of fat digestion. The bile fistula animal was shown to be capable of absorbing a significant quantity of all of the fats studied, except tristearin. The defect in fat absorption was greatest with triolein, 47% of the amount fed was shown to be excreted over and above the amount found in the normal animals. Compared to other fats there was a 37% defect for sodium oleate and a 7% defect for sodium stearate.

From the defects in fat absorption summarized above, it is apparent that the soaps of both the saturated and unsaturated fats were absorbed in the bile deficient state more efficiently and to a significantly greater degree than the corresponding triglycerides. This observation assumes more importance when the controversy over the function of bile and the state of the fat prior to its absorption is recalled. At one time, soap was considered by some to represent the end stage of digestion as put forth in the old Pflueger theory of fat absorption. However, the existance of soaps in the intestine was later denied by Verzar (60), who believed that soaps could not exist in the intestine, and who also thought that fatty acids represented

the end stage of fat digestion. He (60) also put forth the concept that the function of bile acids was not solely limited to the processes of fat digestion, but was concerned with absorptive processes as well.

If either fatty acids or soaps are the end products of fat digestion, and bile acids were solely concerned with digestive processes, it would seem logical that these fats should be absorbed to the same degree in the bile deficient as they are in the normal state, since no preliminary digestion would be necessary. At this point there is no need to enter into the discussion of the fatty acid versus soap controversy because soaps fed to dogs would probably appear in the intestine as soaps mixed with fatty acids, resulting from the hydrolysis of the soaps which would occur in the stomach at a low pH.

From these investigations it appears that this reasoning is not correct, as shown by the significantly lesser degree of soap absorption in the bile deficient as compared to the normal state. From this fact it would seem either that sodium soaps or fatty acids are not necessarily the end products of digestion, or that bile has a function over and above that concerned with the digestive processes. The consideration that bile is not acting solely as an agent of digestion is reasonable, but in no way directly supported by the fact that bile acids are present not only in the intestinal lumen, but also adsorbed to and present in the mucosal cells (48).

With bile acids in such close association with the mucosal cells one could easily visualize the possibility that these acids could possess some function apart from that concerned with digestion, per se.

The lesser magnitude of the absorption defect for soaps compared to triglycerides in the bile deficient animals supports the well known role of bile acids in the digestive process. On the other hand, the significant magnitude of the absorption defect for soaps following bile deprivation may be considered evidence for the role of bile in fat absorption. This latter conclusion results from the consideration that if bile was solely concerned with digestion, the feeding of the end-products of digestion, soaps or fatty acids, should result in the same degree of absorption exhibited in the normal state. Conversely, if absorption of these end-products of digestion is not as complete in the bile fistula compared to the normal animal, bile must be concerned with absorption as well as digestion.

The studies demonstrated very poor absorption for saturated fats ($< 20\%$) are consistent with the previous observations (60) that the highly saturated long chain fats were poorly absorbed in the normal animal (pure stearin 9-14% and sperm oil 15%).

If attention is now focused upon the fecal lipid phosphorous excretion the results indicate that the only

significant finding was in reference to tristearin fed animals. In these animals when bile was diverted from the intestine there was more lipid phosphorous excreted than in the same animals in the normal state. This finding differs from those of previous authors (35). However, these authors presented their results only in the form of preliminary observations. Their results suggested that there was a significant increase in fecal lipid phosphorous excretion in the bile fistula animal, and that unsaturated fats added to the diet further increased the excretion of lipid phosphorous to a greater extent than did the addition of saturated fats.

The data obtained in the investigations presented in the present study would indicate that there was no change in the fecal lipid phosphorous excretion in the bile fistula animals, and that the addition of unsaturated fat to the diet did not modify the lipid phosphorous excretion in either the normal or bile deficient dog. The significance of the increase occurring only in the tristearin feeding experiments and not in the sodium stearate, triolein, or sodium oleate feeding experiments is not clear at the present time. However, the recent observations and investigations into the nature of fecal lipid phosphorous of rats by Swell (58) appears quite pertinent. These investigators found varying increases in fecal lipid phosphorous when various fats were added to the diets of

rats. When they investigated the nature of this phosphorous-containing lipid compound they discovered that it contained no choline. With further investigation it was established to their satisfaction that they were dealing with a calcium-phosphate-fatty acid complex rather than the more conventionally conceived phospholipid, lecithin. This complex was shown to be hydrolyzed with 1 normal HCl, a maneuver which is important to the consideration of the interpretation of the data presented here.

In the method used for the study presented here the extraction of the lipids from fecal material was carried out after acidification with HCl. Thus, the possibility that hydrolization of any calcium-phosphate-fatty acid complex which might have been present with the subsequent loss of lipid-phosphorous can be easily visualized. The determination of phospholipids present in the form of lecithin would have been unaffected since it was shown in the methods section that the extraction procedure related the amounts of egg lecithin added to fecal suspensions linearly to the amounts of lecithin recovery.

SUMMARY AND CONCLUSIONS

Fecal lipid losses have been studied in dogs both before and after removal of bile from the intestinal tract. These dogs were fed a basal "fat-free" diet alone or to which triolein, tristearin, sodium oleate, and sodium stearate were individually added, with the same diet fed to each dog both before and following removal of bile. Fecal aliquots were analyzed quantitatively for total lipid and the lipid characterized chemically in terms of its degree of saturation and its lipid phosphorous content.

Results obtained in this investigation demonstrate a significant absorption of triolein, sodium oleate, and sodium stearate in the bile deficient animal, although in each case absorption was defective compared to the intact animal. This defect in absorption seen in bile fistula dogs appears to be greater in the triolein fed animals than in the ones fed sodium oleate or sodium stearate.

The iodine number of fecal lipids was shown to differ only when saturated fats were added to the diet. As expected the poorly absorbed saturated fats influenced this determination greatly. No differences were noted between animals fed the basal diet exclusively and those fed the basal diet with added amounts of triolein or sodium oleate.

Conclusions Drawn From The Results Include:

1. In the normal state greater than 94% of the fed triolein and sodium oleate were absorbed, but following bile deprivation only 48% of the triolein and 58% of the sodium oleate were absorbed. In the case of sodium stearate fed to normal dogs only 20% was absorbed. This amount decreased to 13% following bile deprivation in the same animals. Only negligible amounts of tristearin were absorbed in either the bile fistula or normal dog. Therefore, no inferences can be drawn concerning the effect of bile deprivation on the absorption of long chain saturated triglycerides.

2. The sodium salts of the fatty acids were more completely absorbed in the bile fistula animal than were the corresponding triglycerides.

3. The bile acids appeared to facilitate the absorption of the long chain unsaturated fats to a greater degree than they did the long chain saturated fats.

4. The bile acids in dogs appear to have an extradigestive function facilitating the mechanism of absorption.

5. No difference could be demonstrated in fecal lipid phosphorous excretion in bile deficient animals as compared to their paired controls except in the tristearin feeding experiments where the removal of bile was shown to significantly elevate fecal lipid phosphorous.

A P P E N D I X

TABLE I

VALIDATION OF TOTAL LIPID EXTRACTION PROCEDURE SHOWING RECOVERY OF ADDED KNOWN AMOUNTS OF LIPID

TRIOLEIN - Extracted Alone

Sample	Amount Added mgm. X	Amount Recovered mgm. Y	Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	25.4	27.0		b_{yx} = 0.9974 ± 0.0031	
2	50.7	52.3			
3	76.1	76.8		$\bar{y}$ = 276.8 ± 0.82	
4	100.2	101.9			
5	150.3	152.5			
6	300.6	301.6			
7	601.3	599.9			
8	902.0	902.2			

TRIOLEIN: Recovery of Aliquots Added to Fecal Suspensions

Sample	Amount Added mgm. X	Amount Recovered mgm. Y	Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	29.7	31.9	22	220.9	217.8
2	29.7	31.9	23	225.9	229.4
3	47.8	47.8	24	227.8	248.2
4	50.3	50.0	25	227.8	245.9
5	51.9	54.7	26	248.1	234.4
6	52.9	55.6	27	335.0	359.3
7	59.2	60.2	28	357.1	408.6
8	53.2	62.9	29	376.8	373.0
9	59.5	61.5	30	376.8	383.7
10	59.5	61.9	31	420.4	420.2
11	99.1	103.5	32	420.4	421.5
12	99.1	103.6			
13	99.8	95.7		b_{yx} = 1.037 ± 0.016	
14	100.1	99.5			
15	101.3	103.7		$\bar{y}$ = 175.6 ± 10.7	
16	103.7	107.0			
17	106.3	110.9		r = 0.9965	
18	113.5	123.5			
19	219.9	269.7			
20	220.0	220.8			
21	220.1	224.1		$P^* < 0.001$	

* Probability of random sampling yielding the same correlation of b_{y1x} to b_{y2x} by chance.

TABLE I CONTINUED

SODIUM OLEATE - Extracted Alone

Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	25.6	20.8
2	51.7	43.7
3	81.0	72.2
4	160.0	144.5
5	304.8	274.5
6	401.3	365.8
7	501.8	452.1
8	710.6	651.8

$b_{yx} = 0.9169 \pm 0.0057$

$\bar{y} = 255.1 \pm 2.6$

SODIUM OLEATE: Recovery of Aliquots Added to Fecal Suspensions

Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	50.0	55.6
2	50.1	43.5
3	91.8	89.6
4	94.0	87.0
5	120.0	110.3
6	120.7	107.5
7	151.2	136.2
8	151.4	136.7
9	173.3	158.3
10	186.9	170.7
11	231.4	216.0
12	233.2	213.2
13	292.7	270.6
14	301.5	273.4
15	331.1	309.1
16	331.3	309.6
17	403.7	365.1
18	400.6	366.0
19	499.9	464.8
20	559.3	522.1

$b_{yx} = 0.9244 \pm 0.0134$

$\bar{y} = 220.3 \pm 4.09$

$r = 0.9995$

$P^* < 0.05$

TABLE I CONTINUED

TRISTEARIN - Extracted Alone

Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	29.0	32.3
2	50.4	52.9
3	75.6	75.6
4	104.8	105.9
5	152.3	155.6

$b_{yx} = 0.9998 \pm 0.0189$

$\bar{y} = 84.5 \pm 0.58$

TRISTEARIN: Recovery of Aliquots Added to Fecal Suspensions

Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	26.1	18.8
2	26.1	21.7
3	51.1	53.1
4	52.5	52.1
5	76.0	74.4
6	77.2	72.0
7	93.8	94.8
8	100.0	102.8
9	149.9	148.3
10	150.4	150.6
11	192.5	190.7
12	196.4	197.8
13	250.9	248.5
14	251.4	244.0
15	301.0	295.5
16	301.5	296.8
17	397.8	401.2
18	399.2	403.4

$b_{yx} = 1.0061 \pm 0.0157$

$\bar{y} = 170.3 \pm 7.8$

$r = 0.9997$

$P^* < 0.001$

TABLE I CONTINUED

SODIUM STEARATE - Extraction Alone

Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	37.5	35.0
2	57.1	53.3
3	74.7	66.2
4	152.8	136.6
5	300.8	273.8
6	407.6	369.0
7	601.0	541.1

$b_{yx} = 0.9012 \pm 0.0033$

$\bar{y} = 210.7 \pm 1.7$

SODIUM STEARATE: Recovery of Aliquots Added to Fecal Suspension

Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	25.4	22.4
2	27.7	23.5
3	50.9	44.4
4	50.9	44.6
5	101.2	88.9
6	102.5	88.7
7	154.4	137.0
8	155.9	136.2
9	230.0	186.9
10	239.6	221.6
11	351.9	308.5
12	355.8	313.0
13	451.0	404.6
14	452.3	400.9
15	545.7	501.9
16	594.3	528.6
17	598.5	548.7

$b_{yx} = 0.9070 \pm 0.0088$

$\bar{y} = 235.3 \pm 7.2$

$r = 0.9993$

$P^* < 0.001$

TABLE II

VALIDATION OF PHOSPHOLIPID EXTRACTION AND DETERMINATION PROCEDURE

Sample	Amount Added mgm. X	Amount Recovered mgm. Y
1	3.0	2.3
2	3.0	2.1
3	5.0	4.0
4	5.0	3.4
5	5.0	3.9
6	10.0	7.9
7	10.0	7.8
8	15.0	10.7
9	15.0	10.9
10	15.0	10.8
11	15.0	11.5
12	20.0	12.9
13	20.0	12.9
14	30.0	21.7
15	30.0	18.9
16	40.0	31.0
17	40.0	32.7
18	50.0	36.6
19	50.0	39.1
20	51.2	38.3
21	51.2	43.7

$b_{yx} = 0.7837 \pm 0.069$

$\bar{y} = 17.3 \pm 1.7$

$r = 0.9926$

$P^* < 0.01$

TABLE III

FECAL FAT EXCRETION - BASAL DIET - NO FAT ADDED

	Dog No.	Wt. Kg.	Fat in Basal Diet gm./D.	Fat Ingested gm./Kg./D	Fecal Fat loss gm./D	Fecal Fat loss gm./Kg./D
Normal	7	12.0	1.545	0.129	4.165	0.347
	13	9.5	1.225	0.129	0.831	0.087
	14	10.4	1.339	0.129	1.332	0.128
	16	10.4	1.339	0.129	0.767	0.074
	18	11.8	1.519	0.129	1.087	0.092
	19	12.0	1.545	0.129	1.045	0.087
	22	12.3	1.581	0.129	1.053	0.086
	24	9.0	1.159	0.129	0.813	0.090
	26	10.6	1.365	0.129	1.547	0.146
	28	12.5	1.607	0.129	1.495	0.120
	29	13.8	1.776	0.129	1.850	0.134
Bile Fistula	7	12.0	1.193	0.099	2.478	0.206
	13	9.5	1.225	0.129	2.238	0.235
	14	10.4	1.339	0.129	2.268	0.258
	14	10.4	1.339	0.129	3.083	0.296
	16	16.4	1.339	0.129	2.492	0.240
	18	11.8	1.519	0.129	2.411	0.204
	19	12.0	1.545	0.129	2.207	0.184
	22	12.3	1.581	0.129	2.385	0.193
	24	9.0	0.915	0.102	1.309	0.145
	26	10.6	1.064	0.100	1.643	0.155
	28	12.5	1.607	0.127	2.848	0.228
	29	13.8	1.764	0.128	2.992	0.217

Paired Comparison $P < 0.02$

TABLE IV

Fecal Fat Excretion - Basal Diet - Triolein Added

	Dog No.	Wt. Kg.	Triolein Ingested gm./D	Triolein Ingested gm./Kg./D	Fecal Fat Loss gm./D	gm./Kg./D	Regression Analysis
Normal	7	12.0	60.0	5.0	1.705	0.142	$b_{yx} = 0.057 \pm 0.010$
	7	12.0	30.0	2.5	1.672	0.139	
	13	9.5	23.7	2.5	1.282	0.135	$\bar{y} = 0.126 \pm 0.096$
	19	12.0	60.0	5.0	1.380	0.115	
	26	10.6	53.0	5.0	2.472	0.229	$r = 0.13$
	28	12.5	62.5	5.0	1.785	0.143	
	29	13.8	69.0	5.0	2.038	0.148	$\bar{y} = 0.004 \pm 0.57\ x$
						0.152 Mean	
Bile Fistula	7	12.0	9.1	0.8	7.613	0.634	$b_{yx} = 0.526 \pm 0.030$
	13	9.5	23.7	2.5	13.539	1.425	
	19	12.0	34.8	2.9	14.974	1.248	$\bar{y} = 0.77 \pm 0.189$
	26	10.6	23.5	2.2	15.800	1.490	
	28	12.5	50.9	4.1	33.155	2.652	$r = 0.9858$
	29	13.8	61.6	4.5	36.226	2.625	$\bar{y} = 0.21 \pm 0.526x$
						1.679 Mean	

Paired Comparison $P < 0.01$

TABLE V

Fecal Fat Excretion - Basal Diet - Sodium Oleate Added

Dog No.	Wt. Kg.	Sodium Oleate Ingested gm./D	Sodium Oleate Ingested gm./Kg./D	Fecal Fat Loss gm./D	Fecal Fat Loss gm./Kg./D	Regression Analysis
7	12.0	27.3	2.1	2.063	0.172	$b_{yx} = 0.0732 \pm 0.0016$
13	9.5	21.6	2.1	1.282	0.135	
19	12.0	50.9	3.9	3.316	0.276	$\bar{y} = 0.221 \pm 0.105$
26	10.6	46.3	4.1	6.350	0.599	$r = 0.7946$
28	12.5	44.7	3.3	6.506	0.520	
29	13.8	67.4	4.5	6.060	0.439	$\bar{y} = 0.129 \pm 0.0732x$
					0.357 Mean	
7	12.0	27.3	2.1	15.527	1.294	$b_{yx} = 0.426 \pm 0.002$
13	9.5	21.6	2.1	8.327	0.876	
19	12.0	22.8	1.8	11.266	0.939	$\bar{y} = 0.57 \pm 0.114$
26	10.6	43.4	3.8	20.853	1.967	$r = 0.9806$
28	12.5	20.1	1.5	6.600	0.528	
29	13.8	38.6	2.6	17.968	1.302	$\bar{y} = 0.199 \pm 0.426x$
					1.151 Mean	

Paired Comparison $P < 0.01$

TABLE VI

Fecal Fat Excretion - Basal Diet - Tristearin Added

	Dog No.	Wt. Kg.	Tristearin Ingested gm./D	Tristearin Ingested gm./Kg./D	Fecal Fat Loss gm./D	Fecal Fat Loss gm./Kg./D	Regression Analysis
Normal	14	10.4	52.0	5.0	47.154	4.534	The data does not include a sufficient range of values to permit this type of analysis.
	16	10.4	52.0	5.0	34.805	3.347	
	18	11.8	59.0	5.0	30.342	2.571	
	22	12.3	61.5	5.0	40.071	3.258	
	24	9.0	45.0	5.0	40.905	4.545	
						3.651 Mean	
Bile Fistula	14	10.4	52.0	5.0	59.667	5.737	
	14	10.4	52.0	5.0	44.492	4.292	
	16	10.4	52.0	5.0	42.843	4.120	
	18	11.8	45.1	3.8	46.235	3.918	
	22	12.3	61.5	5.0	52.543	4.272	
	24	9.0	20.1	2.2	20.709	2.307	
						4.928 Mean	

Paired Comparison $P < 0.15$

TABLE VII

Fecal Fat Excretion - Basal Diet - Sodium Stearate Added

Dog No.	Wt. Kg.	Sodium Stearate Ingested gm./D	Sodium Stearate Ingested gm./Kg./D	Fecal Fat Loss gm./D	Fecal Fat Loss gm./Kg./D	Regression Analysis
14	10.4	52.0	4.5	42.263	4.064	$b_{yx} = 0.799 \pm 0.027$
16	10.4	52.0	4.5	41.532	3.993	
18	11.8	48.7	3.7	25.224	2.138	$\bar{y} = 0.04 \pm 0.799x$
22	12.3	66.0	4.9	51.000	4.146	
24	9.0	31.5	3.2	24.773	2.752	$\bar{y} = 1.16 \pm 0.209$
						$r = 0.9855$
					3.419 Mean	
14	10.4	56.0	4.9	47.387	4.556	$b_{yx} = 0.871 \pm 0.016$
16	10.4	50.1	4.4	40.592	3.903	
18	11.8	33.9	3.0	23.147	1.962	$\bar{y} = 0.21 \pm 0.871x$
22	12.3	55.8	4.1	45.520	3.700	
24	9.0	39.0	3.0	33.655	3.793	$\bar{y} = 1.26 \pm 0.108$
						$r = 0.9799$
					3.583 Mean	

Paired Comparison $P < 0.075$

TABLE VIII

Dog No.	Type Fat Added to Basal Diet	Mean	Fecal Lipid Phosphorous		Paired Comparison "P" Value	Iodine Number		Paired Comparison "P" Value
			Normal	Bile Fistula		Normal	Bile Fistula	
7			0.90	0.51		58.4	40.4	
13			0.28	0.75		53.0	38.3	
14			0.50	0.57		48.9	38.2	
16			0.16	0.38		38.4	40.0	
18			0.22	0.66		36.2	40.6	
19	None		0.25	0.37		24.0	33.1	
22			0.27	0.50		27.6	30.0	
24			0.40	0.33		36.3	41.9	
26			0.62	0.27		42.0	38.8	
28			0.42	7.37		46.6	39.8	
29			0.50	0.40		31.8	34.8	
		Mean	0.40	1.10(0.47)	0.25	40.4	37.7	0.40
7			0.36	0.36		51.5	43.2	
13			0.24	0.28		41.3	21.2	
19			0.19	0.30		43.9	15.0	
26	Triolein		0.57	0.31		48.1	51.5	
28			0.48	0.40		57.6	45.4	
29			0.42	0.63		55.6	44.3	
		Mean	0.38	0.46	0.50	49.7	36.8	0.25
7			0.31	0.38		48.3	64.3	
13			0.24	0.64		41.3	49.0	
19			0.24	0.36		46.4	37.5	
26	Sodium Oleate		0.60	0.77		57.2	48.3	
28			0.33	0.29		52.9	50.3	
29			0.75	0.52		48.6	71.9	
		Mean	0.41	0.49	0.70	48.2	64.3	0.20

TABLE VIII CONTINUED

Dog No.	Type Fat Added to Basal Diet	Mean	Fecal Lipid Phosphorous		Paired Comparison "P" Value	Iodine Number		Paired Comparison "P" Value
			Normal	Bile Fistula		Normal	Bile Fistula	
14	Tristearin		0.19	0.35		3.6	4.9	
16			0.54	0.47		5.6	5.7	
18			0.19	0.47		4.6	5.2	
22			0.22	0.49		4.2	4.8	
24			0.30	0.40		3.6	3.7	
		Mean	0.29	0.47	0.001	4.3	4.8	0.30
14	Sodium Stearate		0.20	0.35		1.9	2.9	
16			0.44	0.48		2.5	3.2	
18			0.16	0.37		2.1	5.3	
22			0.61	0.63		2.2	3.2	
24			0.41	0.67		2.7	2.9	
		Mean	0.37	0.50	0.40	2.3	3.5	0.30

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