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THE POSTNATAL PIG.

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DEVELOPMENT OF EXOCRINE SECRETION OF THE PANCREAS  
AND NUTRIENT UTILIZATION IN THE POSTNATAL PIG

by

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## INTRODUCTION

Evidence is presented in the "Review of Literature" section of this dissertation which has led investigators to the judgment that the digestive system undergoes changes during the postnatal period. Some of these changes have been characterized, others are not fully understood. Also evidence is presented which suggests that the composition of a diet influences the digestive functions of the animal consuming the diet. Therefore, investigations into the digestive function of newborn animals should be conducted with the intention of distinguishing between the natural physiological development and function of the digestive organs and dietary influences upon the development and function of the digestive organs.

Regarding the baby pig, the experimental animal used throughout the research program to be reported herein, evidence accumulated indicates that the baby pig's digestive system undergoes characteristic changes from birth to 6 or 7 weeks of age; that the growth rate and feed efficiency of pigs fed a diet in which the protein is supplied primarily by soybean meal is generally inferior to that of pigs fed an isonitrogenous diet in which the protein is supplied primarily by dried skim milk; and further that this performance differential becomes smaller and smaller as the animals approach 8 weeks of age. The evidence accumulated in previous investigations indicates that the reduction of the performance differential for the two diets can be accredited to improved utilization of the soybean diet whereas utilization of the dried skim milk diet remains relatively unchanged.

The research program leading to the preparation of this dissertation was centered around investigations of the digestive function of the pancreas gland of the postnatal pig between 2 and 8 weeks of age. The proposed research outline was based on a series of investigations involving the analysis of the exocrine pancreatic secretion collected from a pancreatic fistula and comparisons of nutrient digestibility of pigs with ligated pancreatic ducts and sham operated control animals. The pancreozymin-secretin pancreatic function test was also applied.

## REVIEW OF LITERATURE

The exigency of this study is indicated by the evidence accumulated in baby pig nutrition investigations which indicate that the utilization of a soybean basal diet improves steadily with advancing age from a low point at birth toward a plateau at the age of about 8 weeks, whereas the utilization of a dried skim milk basal diet is comparatively high shortly after birth and undergoes no appreciable change with advancing age.

Physiological experiments support a dietary influence on the physiological functions of the exocrine pancreas. In view of this it seemed pertinent to review the literature relating these differences in dietary utilization by the baby pig, changes in the digestive system of the young animal with advancing age, and dietary influences on the function of the digestive glands and organs. The literature will be primarily limited to studies on the pancreas rather than the whole digestive system since the pancreas was selected for this study.

A comprehensive search of the literature revealed no experimental studies on the physiological performance of the pancreas of the pig. This emphasizes the need for further research in this area and for the purposes of this review makes it necessary to rely entirely on investigations with other species.

Effect of Age and Diet on Nutrient Utilization and  
Growth Performance of the Early Postnatal Pig

Results of the many studies conducted at the Iowa State University, Swine Nutrition Research Farm were summarized by Catron et al. (15).

He stated that there is no question that the baby pig is deficient of certain digestive enzymes up to 5 weeks of age and emphasized that the diets of these pigs should be supplemented with digestive enzymes with a particular emphasis on pepsin. He also cited evidence that baby pigs fed a diet in which dried skim milk was the source of dietary protein made significantly faster gains on less feed per pound of gain than pigs fed either Drackett protein or a diet in which soybean meal was the major source of protein. In a separate publication a year later Catron (14) stated that dried skim milk is an essential ingredient in the diets for baby pigs weaned at an early age if maximum performance is to be expected. He presented experimental evidence that both the casein and lactose fractions of dried skim milk were responsible for this greater performance.

Baker (8) made several comparisons of dried skim milk and soybean meal in baby pig diets and in most every case the efficiency of feed utilization and growth was greater for pigs fed the dried skim milk.

Peo et al. (114) established by use of the depletion-repletion technique that baby pigs repleted with a dried skim milk base diet made more rapid repletion gains on less feed per pound of repletion gain than similar pigs repleted with a soybean meal base diet with or without 0.1 percent of dl-methionine supplement.

Hays et al. (55) reported that 10-day-old pigs fed a dried skim milk basal diet (20 percent protein) for a period of 5 weeks gained 25.8 pounds on 1.58 pounds of feed per pound of gain. Similar pigs fed a soybean meal basal diet (also 20 percent protein) for the same period gained 11.0 pounds on 2.34 pounds of feed per pound of gain. Supplementation of the

soybean meal diet of another similar group of animals with 0.05 percent methionine improved gains and efficiency of feed utilization so that the performance approached that of animals fed the dried skim milk diet.

Lucas and Lodge (87) reported that liquid diets are required for pigs weaned at 2 days of age, and that these pigs are unable to utilize starch, sucrose, or protein from sources other than milk.

Crampton (18) compared growth and efficiency of feed utilization of pigs between 29 and 75 pounds of body weight on a dried skim milk diet and a tankage, fishmeal, linseed oil meal, mineral mixture diet and found no appreciable difference.

Crane (19) reported that a diet containing 40 percent dried skim milk was superior to diets with 0, 13, or 20 percent dried skim milk for pigs weaned under 10 pounds body weight, and further that 10 percent fat was superior to 7.5, 12.5, or 15.0 percent fat. Hudman (63,65) demonstrated that a 40 percent dried skim milk diet was superior to diets with 0 or 20 percent dried skim milk and that both the casein and lactose fraction of dried skim milk independently improved the diets and that the effects of each were synergistic when added in combination so that the performance of animals consuming a diet with both approached that of the 40 percent dried skim milk diet.

Lewis (82) compared several commercial sources of purified soybean protein in baby pig diets, and found that animal growth and feed efficiency varied considerably among sources. All the soybean proteins, supplemented with methionine, were inferior to a diet in which the protein was supplied by dried skim milk.

Hudman (65,63) evaluated a variety of carbohydrates in an effort to improve the diets for baby pigs. He weaned pigs at 3, 6, 9, 12, 15, and 18 days of age onto a diet in which corn starch was the sole source of carbohydrate. There was no significant difference in the total 35 day gains of these animals except for those pigs weaned at 3 days which averaged about 2 pounds less weight than all the others. He also found a significant linear improvement in the 14 day body weight gains of pigs weaned at 7 days onto experimental diets as the dried skim milk was increased from 0 to 20 to 40 percent of the diet. He also compared 11 carbohydrate sources using a soybean meal basal diet for baby pigs between the ages of 7 and 35 days. Lactose produced significantly better gains than corn starch, cooked corn starch, amidex, corn syrup solids with a dextrose equivalent of 24, ground oat groats, or corn flakes.

Roy (120) indicated that milk is necessary in the diet of early weaned calves if satisfactory weight gains are to be obtained.

The evidence presented above indicates that dried skim milk is superior to soybean meal as a source of dietary protein for baby pigs. It is apparent that the carbohydrate fraction of dried skim milk contributes to this superiority as well as the protein fraction.

#### Evidence of Digestive Inadequacy in the Early Postnatal Pig and Subsequent Development of the Digestive System

In 1957, Catron (15) expressed the opinion that since it is well known that satisfactory growth and feed utilization can be obtained with pigs older than 5 to 8 weeks of age on a soybean meal diet, the poor performance of pigs under 5 weeks of age cannot be explained on an amino

acid imbalance basis, nor on the basis of other known or unidentified growth factors, indicating to him a digestive enzyme insufficiency. He outlined the four major experimental approaches followed in attempts to verify this hypothesis, which consisted of; assaying the enzyme activity of tissues of the digestive glands removed from pigs sacrificed at weekly intervals from birth to 6 weeks of age; supplementation of baby pig diets with a selection of enzyme preparations containing protease or amylase activity or both; feeding hydrolyzed soybean meal to baby pigs at varying degrees of hydrolysis; and lastly to appraise the factors affecting variation in baby pig responses to dietary enzyme supplementation. The same author in a separate report (14) stated that the baby pigs' mild digestive enzyme system is capable of breaking down milk protein, but not vegetable protein during the first five weeks of life.

#### Dietary enzyme supplementation and dietary hydrolysates

Davison (27) reported that 22 infants with digestive disturbances were given 85,000 to 7,500,000 amylase units and from 990 to 2,160 trypsin units daily for 3 to 16 days. Of the 22 infants, 16 gained weight, showed improved appetite and a reduction of number of stools. Upon removal and analysis of duodenal contents, they found amylase activity and concluded that gastric digestion did not destroy the amylase.

Catron (13) reported that in 13 separate experiments, involving 895 pigs, supplementation of baby pig diets with various levels of pepsin produced favorable responses in baby pig growth. He further indicated that supplementing baby pig diets with pepsin at the rate of 1.0 percent

has yielded an average improvement in gains of 6.0 percent and in feed efficiency of 4.0 percent.

Lewis et al. (83) reported that baby pig gains and feed efficiency were increased as much as 29 and 23 percent respectively by proteolytic enzyme supplementation of soybean protein and casein basal diets. The enzymes used in these investigations were pancreatin, pepsin, Star-Zyme P, and Papain. A special Drackett protein of low molecular weight reduced gain and feed efficiency markedly. Lewis et al. (84) in a separate report, apparently based largely on the same investigations reviewed immediately above, indicated that proteolytic enzyme supplementation of a soybean protein diet containing lactose did not yield a response when fed to older pigs. Lewis (82) reported that supplementation of a baby pig diet with both pancreatin and pepsin resulted in better animal performance than when either was fed alone, and that supplementation of the diets of baby pigs, maintained in individual pens in a relatively disease-free building, with proteolytic enzymes failed to produce a consistent improvement in animal performance. He further reported that predigestion of solvent extracted soybean meal or of Drackett C-1 assay soybean protein with pancreatin resulted in improved pig growth and feed utilization when fed to baby pigs, and that increasing the extent of hydrolysis of the proteins resulted in corresponding improvements in animal performance.

Baker (8) and Neagle (107), who have carried these investigations further used the same facilities and their experimental animals were offspring from the same swine herd as was used by Lewis (82,83,84). Baker (8) conducted 15 experiments involving 2006 pigs in an attempt to

determine; the ability of the baby pig to utilize soybean protein, the adequacy of pepsin secretion of the baby pig, and factors which cause variations in the response of baby pigs receiving supplemental pepsin in their diet. Five of 6 commercial enzyme preparations, all with proteolytic activity, some with amylolytic activity, when added to a Drackett protein-lactose diet improved both growth and feed efficiency of baby pigs. In one particular experiment the best gains and feed efficiency were obtained by addition of inactivated pepsin to the diet and the addition of activated pepsin to a dried skim milk diet resulted in poorer growth and feed efficiency. In another individual experiment pepsin was added to both soybean and dried skim milk diets at levels of 0, 0.25, 0.50, and 1.0 percent. Although there were no statistically significant differences in animal performance on these diets, gains were slightly greater and feed efficiency poorer on the pepsin supplemented soybean diets, whereas both growth rate and feed efficiency were poorer after the addition of pepsin to the dried skim milk diets. In still another experiment, pepsin was added to the diet of heavy and light pigs. It was found that the heavy pigs grew faster and more efficiently than the light pigs. Pepsin supplementation did not alter the performance of the heavy pigs but improved both gain and feed efficiency of the light pigs. In another separate experiment baby pigs gained faster on the soybean meal basal diet than on a dried skim milk diet and the efficiencies were essentially the same. Supplementing both of these basal diets with pancreatin or pepsin or a combination of both impaired growth rate and feed efficiency in every case. In another experiment he observed that pepsin supplementation

improved the soybean basal diet but impaired the Drackett protein basal diet. Another experiment revealed that pigs weaned at 1 week of age responded to supplemental pepsin whereas pigs weaned at 3 weeks of age did not. In other experiments evidence was obtained that pepsin supplementation at a rate of 0.25 percent improved animal performance on a 15 percent protein diet but not on 20 and 25 percent protein diets and further that the 20 percent protein diet with no pepsin yielded the highest protein digestion coefficient when fed to 2-week-old pigs. After the pigs reached 4 weeks of age the digestion coefficients were similar for all protein levels. Pepsin increased the protein digestion coefficient for all protein levels when added to diets of baby pigs at 2 weeks of age but had negligible effects at 4 weeks of age. He also reported two experiments in which he used soybean meal hydrolysates as a partial source of dietary protein. From these studies he concluded that predigestion with ficin to 17.5 percent soluble nitrogen showed the most promise in improving the performance of baby pigs.

Neagle (107) studied the effects of supplementing corn-soybean meal diets with proteolytic enzymes of bacterial and fungal origin and the predigestion of soybean protein on baby pig performance. He conducted 10 experiments involving 567 pigs. In summary he stated that the addition of pepsin, Pabst L-56-D, Pabst L-276, Rhozyme B-6, and Rhozyme P-11, each at specific levels, improved baby pig gains, but not feed efficiency. Pabst L-56-D did not improve the apparent digestibility of protein in one study. Baby pig gains and feed efficiency were impaired by feeding soybean meal predigested for 3, 5, and 7 hours with 2.0 percent Protease 30. Animal

performance was similarly and significantly reduced by feeding soybean meal predigested for 2, 6, and 9 hours with 2.0 percent pancreatin. Soybean meal predigested with 2.0 percent Pabst L-56-D for 1, 6, and 9 hours improved gains and feed efficiency slightly when fed to baby pigs. The predigestion did not noticeably affect the apparent digestibility of the protein. Predigestion of raw soybean flour with pancreatin significantly improved baby pig performance when incorporated in their diets. The magnitude of the response was proportional to the degree of antitrypsin destruction. Where all the antitrypsin activity was destroyed the animal performance was still inferior to the performance of animals receiving a diet containing toasted soybean meal. A similar experiment in which Pabst L-56-D was used instead of pancreatin and the predigestion period continued for 9 hours, significantly improved gains, feed efficiency and apparent digestibility of the dietary protein. The antitrypsin activity was destroyed. The apparent digestibility was higher and the rate of gain and feed efficiency were poorer for the pigs fed the raw soybean flour hydrolysate than pigs fed a soybean meal or soybean meal hydrolysate diet. Considerable enzymatic activity was found in the hydrolysate as added to some of the diets.

Hudman (63) found that there was a significant increase in baby pig gains as the dextrose equivalent of six different carbohydrates, all products of the corn milling industry, increased from 10 to 42. Cooking corn starch to a dextrose equivalent of 10 significantly reduced gains, but further cooking produced corresponding increases in gains. He also reported that sucrose produced statistically greater gains for baby pigs

to 5 weeks of age than eight other carbohydrates when compared in a casein protein basal baby pig diet. Lactose, dextrose and dextrimaltose produced comparable gains and feed efficiencies and the gains were significantly greater than was produced by ground yellow corn, ground oats groats and amidex.

Cunningham and Brisson (22) reported that 2-day-old pigs fed a liquid diet containing various amylases (pancreatic amylase, malt amylase and pancreatin) at a rate of 1.0 percent of the starch of the diet did not improve starch digestibility. Their data revealed that the control animals had a digestibility of 99 percent and thus an improvement would seem difficult to accomplish or measure. They stated that cooked starch is inferior to raw starch for baby pigs. The same investigators (23) conducted experiments with baby pigs weaned at 2 days of age to determine the effect of supplementing or predigesting diets containing plant and animal proteins with proteolytic enzymes and concluded that enzyme supplementation had no effect on feed consumption or protein digestibility and that predigestion of the dietary proteins caused severe scours. It should be emphasized that in the first trial of this study in which a fishmeal diet was supplemented with pepsin and pancreatin, none of the pigs including the control animals lived past five weeks of age. In the second trial a soybean meal basal diet was supplemented with 1.0 percent pepsin or 1.0 percent inactivated pepsin. The protein digestibility was slightly higher for the control group of animals than for the animals receiving either of the supplemented diets and there was a gradual increase in digestibility throughout the experimental period. There was essentially no difference

in protein digestibility between the groups receiving the active or inactive pepsin. In the third trial of the same study one group of animals received a diet which contained 1.0 percent active pepsin and another group received a diet which was predigested with 1.0 percent active pepsin. All the animals that received the predigested diet developed severe diarrhea and succumbed in 7 days.

Cunningham (21) studied the digestion of starch and some of its degradation products by newborn pigs. He reported that glucose, maltose and a soluble starch preparation were digested more rapidly than raw starch. The rate of digestion of raw starch introduced directly into the intestine was not improved with supplemental pancreatic amylase. He expressed the opinion that his data indicate that the prime factor restricting the digestion of raw starch by newborn pigs is that responsible for the initial rupture of the starch granule and that the rate of digestion of soluble starch is just below that of maltose, and that it is difficult to determine if the rate of digestion is limited by the availability of amylase or maltase.

#### Nutrient digestibility

Gross changes in the digestive system of the young animal have been proposed by a number of investigators. Studies of dietary nutrient digestibility may be considered as speculative verification in support or contrariety of this hypothesis.

Hays et al. (55) observed the changes that occur in apparent protein and dry matter digestibility, nitrogen retention and protein biological

value for baby pigs between 2 and 5 weeks of age. The results of this study indicated that protein and dry matter digestibility were high for pigs at 2 weeks of age and increased only slightly to 5 weeks of age when fed a dried skim milk diet. By comparison the apparent digestibility of protein and dry matter was low for pigs fed the soybean meal diet at 2 weeks of age and increased markedly up to 5 weeks of age, but did not equal that of the dried skim milk fed pigs. The percent nitrogen retention and biological value of protein were low at 2 weeks of age for the pigs fed the soybean meal diet relative to those fed the dried skim milk diet whereas the values were essentially the same at 5 weeks of age. Unlike the soybean meal fed pigs, the nitrogen retention and protein biological value for the pigs fed dried skim milk decreased from a high value at 2 weeks of age to values very similar to those calculated for the soybean meal fed pigs at 5 weeks of age. Supplementation of the soybean meal diet with either arginine or methionine or both in combination, and the dried skim milk diet with arginine had no appreciable affect on protein and dry matter digestibility, nitrogen retention, or protein biological value. Supplementation of the soybean diet with methionine alone stimulated the growth rate and feed efficiency of the animals consuming it.

Lloyd et al. (86) studied the changes that occur in nutrient digestibility by baby pigs between 3 and 7 weeks of age. In the first trial a ration was used which contained 37 percent dried skim milk, 10 percent soybean meal, and 10 percent fishmeal as the primary sources of protein. The apparent digestibility coefficients for each of the nutrients measured

were as follows at 3 and 7 weeks of age respectively: dry matter, 84.4 and 85.9; crude protein, 85.3 and 88.5; total calories, 85.8 and 88.6; ether extract, 39.1 and 55.1; total carbohydrate, 90.1 and 91.1. In a second trial, 13 different fats or oils were included in separate diets at a rate of 20 percent. It was found that the digestibility of crude protein, dry matter, calories, ether extract, and total carbohydrate, with one exception, was greater at 7 weeks of age than at 3 weeks regardless of the fatty acid chain length.

Neagle (107) observed that the apparent digestibility of protein was significantly higher for 5-week-old pigs than for 3-week-old pigs, and significantly higher for pigs fed 20 percent protein diets than for pigs fed 15 percent protein diets.

Cunningham and Brisson (24) used 1-day-old pigs on a liquid diet containing considerable glucose and compared it to a similar diet with an equal amount of maltose. They found that the total carbohydrate digestibility for the pigs on the glucose diets was 99.9 percent and for the maltose fed pigs it was 97.4 percent. In the same study they observed by using isolated loops of baby pig intestines that the carbohydrate digestibility increased with age and that the rate of maltose absorption from the isolated loops increased with age. Cunningham (21) stated that the rate of digestion of soluble starch, maltose, and glucose by newborn pigs would appear to be sufficiently high to meet a large proportion of the pigs energy requirement although consumption of such quantities may cause diarrhea.

Gupta et al. (44) studied the digestion of defatted beef, alcohol extracted casein, and zein by feeding these proteins to rats mixed in a complete diet. Rats were sacrificed at uniform intervals after they had eaten. A comparison diet was prepared which contained an essential amino acid mixture. Their results indicated that beef, casein, and the amino acid mixture were removed from the stomach and small intestine at almost identical rates. Zein removal from the stomach paralleled the other diets for the first two hours, after which time the rate decreased. The percent nitrogen in contents of the small intestine of rats fed zein was approximately 3 to 5 times that of the other diets indicating a slow removal rate. They reported that considerable nitrogen was found in the intestines and stomach of rats fed a protein free diet. They concluded that the failure of free amino acids to disappear from the gastrointestinal tract more rapidly than intact casein or beef proteins suggests that the rate of protein digestion becomes a limiting factor in protein utilization only when the protein consumed is relatively insoluble as is the case with zein.

#### Digestive enzyme activity of glandular tissues, glandular secretions, and ingesta

At the inception of the twentieth century, Mendel (102) reported that lactase activity was measured in the very early embryonic pig and that sucrase activity could not be detected, even in large pig embryos. Sucrase, maltase and lactase were present in the suckling pig but in the adult pig there was no measurable lactase in the intestine although sucrase was always present. He could not demonstrate the presence of pepsin or rennin in embryonic pigs, not even in 11 inch embryos. He stated

that the dog is very similar to the pig, both in the embryonic and adult stages.

Keene and Hewer (71) reported that pancreatic trypsin was present in the human fetus at 16 weeks gestation and showed full development at birth. Klumpp and Neale (75) reported that amylase activity in duodenal contents of infants is low during the first year of age.

Werner (142) studied the peptic and tryptic capacity of the digestive glands of nonsurvival premature infants and full term infants and concluded: that premature infants under 2,500 grams of weight have only few pepsinogen granules in the gastric tissues; full term infants over 3000 grams usually have far greater numbers of granules in the tissues of the digestive glands than premature infants; differences in the tissues between the premature and full term infants are of a greater magnitude in pancreatic tissue than in the gastric mucosa; histological findings in the pancreas were in good agreement with chemically determined activities in the tissue; and that the premature infant is poorly equipped for protein digestion.

Vazquez (132) studied pancreatic function in infants by collecting the duodenal juice after an injection of Doryl, by passing a tube down the alimentary canal to the approximate site at which the pancreas secretes into the duodenum. Samples of duodenal juice were collected from 15 infants, who weighed between 2.9 and 4.2 kilograms, at 1, 3, 5, 7, 9, 15 and 21 days of life, but not always from the same children. Trypsin was always present, but at first in widely varying concentrations. In those cases where the initial trypsin concentration was low, there was an

increase during the following 3 weeks approximating those normally observed at 3 months. Lipase was low during the first 3 days relative to later concentrations which increased to 3 weeks of age. Amylase was low, when present, and in many cases did not appear until about 15 or 20 days of age.

Madey and Dancis (92) examined the duodenal contents of 16 premature infants and found it to contain normal concentrations of trypsin. The gain in weight of infants whose diet was supplemented with casein hydrolysate was found to be no better than that of infants supplemented with whole casein. They concluded that it is unnecessary to supplement the diet of the premature infant with hydrolyzed protein.

Anderson (3) studied the pancreatic enzymes in duodenal juice of infants with the celiac syndrome. He concluded that pancreatic amylase was not a valid indicator of pancreatic function until the age of 8 to 12 months.

Driscoll and Hsia (31) have reviewed the evidence relating to the development of enzyme systems during early infancy. The evidence accumulated suggests that gastric digestion is not as adequate in newborn and premature infants as in the adult human, nevertheless, free acid and pepsinogen granules have been found before birth and salivary ptyalin in the 16-week-embryo. Protease activity has been found in extracts of duodenal tissue from 16-week-embryos and full term infants. Pancreatic digestion does not appear to be fully developed at birth. Pancreatic amylase and lipase are relatively low during the first postnatal year. Gyorgy (48) stated that the newborn, and especially the prematurely-born

infant, is at birth not fully endowed with all the metabolic equipment which characterizes later age periods, and further that the renal function does not reach its maturity until the age of 4 years.

Platt (116) stated that pigs fed a low protein diet or a low protein diet with excessive carbohydrates added had fewer zymogen granules in the pancreas than normally fed pigs.

Kvansnitskii and Bakeevna (79) observed that gastric secretion, collected by way of a gastric fistula, from pigs during their first few days of life contained pepsin and rennase from the first day, but hydrochloric acid did not appear until 20 or 30 days of age. The acid secretion did not increase progressively with advancing age and became very scarce before weaning. They concluded that although pepsin is elaborated early in life it is not active until the hydrochloric acid appears which does not come into full operation until about 2 months of age. They indicated that in vitro tests suggest that pepsin digestion attains an appreciable role at about 45 days of age.

Kitts et al. (74) have studied the development of the digestive enzyme system of the pig during its preweaning phase of growth. They reported that the amylase activity of crude aqueous extracts of the pancreas of suckling pigs increased markedly with advancing age. The lipase activity of the extract was relatively high at birth, and remained high as growth proceeded. Bailey et al. (7) representing the same team of investigators as above (74) found that intestinal lactase activity is high from birth to 2 weeks of age, followed by a precipitous decline to 3 or 4 weeks of age. Intestinal sucrase and maltase increased from negligible

amounts at birth to a maximum at about 25 days of age. Enzyme concentrations were expressed as activity units per kilogram body weight.

Hudman et al. (64) reported that pancreatic amylase activity of baby pig pancreatic tissue increased from birth to 35 days of age. They observed a very marked drop in pancreatic amylase activity from 35 to 42 days of age. They found that pancreatic tissue maltase activity is low at birth and remains low to 42 days of age and also that salivary amylase activity of saliva decreased from birth to 49 days of age.

Lewis et al. (85) sacrificed pigs at various intervals after birth and determined the pepsin and trypsin activity of the dried stomach and pancreas tissue respectively. Pepsin activity of stomach tissue increased markedly from birth to 42 days of age, whereas the trypsin activity of pancreatic tissue varied erratically with advancing age.

Baker (8) reported that the analysis of stomach tissues of baby pigs for pepsin revealed a significant increase in activity per gram of tissue from birth to 8 weeks of age, but that no significant differences were observed in the pepsin activity of gastric ingesta of the same pigs.

Walker (136) reports that the pH of the stomach and duodenum of the baby pig sacrificed 2 hours after suckling is 3.5 and 6.5 respectively. In a separate report (137) the same author gave evidence that pancreatic amylase (activity/gram dry tissue) increased with age after a temporary drop at 2 or 3 weeks of age. Intestinal sucrase and maltase activity increased per gram of dry tissue whereas intestinal lactase and amylase generally decreased with advancing age. Lactase activity was highest at birth and decreased markedly to 2 weeks of age, and then continued to

decrease more gradually to 5 weeks of age. Intestinal amylase activity increased from a low concentration at birth to a maximum at 1 week of age, followed by a gradual decrease to 5 weeks of age. It is significant that in the latter study the enzyme activities were also expressed as total organ activity as well as activity per gram of tissue. The results summarized in these two ways lead to different interpretations. When the total tissue activity was calculated it was found that pancreatic amylase, intestinal maltase and sucrase increased linearly from birth to 5 weeks of age and did not suggest that a maximum was reached. Small intestine amylase activity increased from a very low value at birth to a high plateau at about 1 week of age and varied about the plateau throughout the 5 week period. Total intestinal lactase activity remained remarkably constant from birth to 5 weeks of age.

Hartman et al. (54) evaluated the protease, amylase, and lipase activity of the various digestive organs and their contents of weaned (weaned at 1 week of age) and unweaned baby pigs by sacrificing animals at weekly intervals from birth to 8 weeks of age. They reported that the protease activity per gram of wet stomach tissue was relatively low during the first 2 weeks after birth followed by a steady increase to 8 weeks of age with no significant difference between weaned or unweaned pigs. The protease activity per gram of wet stomach ingesta decreased for weaned and unweaned pigs during the first 3 weeks after birth. This initial drop in the protease activity of stomach ingesta was followed by a continued decrease for the weaned pigs and a marked increase for the unweaned pigs. These results are confounded however by the fact that the total stomach

tissue weight of the weaned pigs increased with advancing age at nearly twice the rate of unweaned pigs and further that the total weight of the stomach ingesta of the weaned pigs was more than twice as great during the 8 week investigation period except for the first 3 weeks during which the quantity of contents were very similar. Pancreatic tissue tributyrinase activity per gram of wet tissue was reported to be high at birth and increased gradually with advancing age for unweaned pigs but the tributyrinase activity dropped at the time the weaned animals were separated from the sow and then increased rapidly to 8 weeks of age to concentrations very similar to those of the unweaned pigs. The total pancreas weights for the weaned and unweaned pigs were similar throughout the 8 week investigation period. Pancreatic amylase activity per gram of wet tissue increased with advancing age and the data yielded a significant linear regression of activity on age. There was no significant difference between the weaned and unweaned groups of pigs. Pancreatic protease activity per gram of wet tissue increased four-fold from birth to 8 weeks of age for the unweaned pigs, whereas it remained relatively low for the first 4 weeks for the weaned pigs and then steadily increased about three-fold to 8 weeks of age. The small intestines of both groups of animals were removed and the ingesta recovered. There was considerably more ingesta in the small intestine of the weaned pigs than the unweaned pigs at each weekly period. Lactase was reported to be the most predominant carbohydrase in intestinal tissue and it was found to decrease from the highest concentration per gram of wet tissue at birth to a plateau at 3 weeks of age and then remained relatively constant throughout the remaining

5 week period. There was no apparent difference between the weaned and the unweaned pigs. The total tissue weights of the small intestines were similar for the two groups of animals from birth to 5 weeks of age, however, from 5 to 8 weeks of age the weaned pigs had more intestinal tissue and ingesta than the unweaned pigs. They also reported that the protease activity per gram of small intestine ingesta increased steadily from birth to 8 weeks of age. The protease activity of the small intestine ingesta was considerably greater for the weaned pigs than the unweaned pigs. Both maltase and sucrase activity per gram of small intestine tissue increased in a linear fashion from a low at birth to a high at 7 weeks of age and there was no appreciable difference between the weaned or unweaned pigs.

They also determined the pH of the contents of the stomach, three sections of the small intestine, and the large intestine. The pH of the stomach varied between 3.3 and 5.2; anterior third of small intestine, 5.8 and 6.5; middle third of small intestine 5.7 and 6.8; posterior third of small intestine, 5.9 and 7.0; and the large intestine varied between pH 5.7 and 7.1. In general the pH of the small intestine was slightly lower for weaned pigs than suckling pigs. The data suggest that there was a slight drop in the pH of the stomach, anterior small intestine, and large intestine as the animals grew older.

Eastman (32) determined the pH of gastrointestinal contents of 455 albino rats between 3 weeks and  $1\frac{1}{2}$  years of age. She reported that the pH of the tract anterior to a selected level has negligible influence on the pH at the selected level and that young rats had a higher pH at all levels of the gastrointestinal tract than older rats, particularly the stomach.

Walker (138) reported that total pancreatic amylase and total small intestinal amylase activity were low at 5 days of age and increased only slightly to 35 days of age. Small intestine lactase and maltase activity (total) was found to be constantly low from 1 to 5 weeks of age. Sucrase activity was not detected in the small intestine tissues or contents of any of the 12 lambs studied. He concluded that the lamb must depend on the early development of the rumen for utilization of all carbohydrates except lactose and glucose. In another publication (139) he reported that the protease activity of the tissues and contents of the digestive organs of the same 12 lambs used in the above report (138) was determined at pH 1.8, 3.5 and 8.5. He found that only the abomasum tissue and contents had protease activity at pH 1.8, all the tissues of the abomasum, small intestine, and pancreas, and the contents of the abomasum and small intestine had protease activity at pH 3.5. Only small intestine tissue and its contents had protease activity at pH 8.5, except for four lambs with pancreatic activity at pH 8.5. The total protease activity of the pancreas measured at pH 3.5 was relatively low and constant from 1 to 4 weeks of age and increased markedly to the fifth week. Total small intestine protease activity (at pH 3.5) increased linearly about five-fold from 1 to 5 weeks of age. The total protease activity of the abomasum tissue (measured at pH 1.8, and 3.5) increased from 1 to 2 weeks of age and then decreased to 5 weeks of age, however the total activity of the abomasum contents increased steadily from 1 to 5 weeks of age.

Indirect studies of pancreatic digestion in the young

Baggenstoss et al. (6) acknowledged the possibility that the fibro-cystic disease of the pancreas of infants could be due to a deficiency of secretin. They could not detect secretin in individual newborns. They did however detect secretin activity by pooling intestinal extracts of three premature infants and also a pooled extract of three full term infants.

West et al. (143) studied changes in the blood amino nitrogen level following ingestion of proteins and protein hydrolysate in infants with normal and deficient pancreatic function. They reported that the blood amino nitrogen level rose 1.0 milligram per 100 milliliters blood  $1\frac{1}{4}$  hour after ingestion of 1.5 gram casein per kilogram body weight by normal and sick children without pancreatic malfunction. An equivalent amount of gelatin under the same conditions caused the blood amino nitrogen to rise 2.0 milligram per 100 milliliters of blood. Blood amino nitrogen concentrations of children with cystic fibrosis of the pancreas did not yield the typical response observed in normal children following ingestion of casein and gelatin, however in a few cases a slight rise occurred about 5 hours after the proteins were ingested. Analysis of duodenal juice collected from these children revealed a lower concentration of trypsin for the children with cystic fibrosis of the pancreas than for normal children. Feeding the acid hydrolysate of casein and gelatin caused greater rises in blood amino nitrogen than the intact proteins. The increase of blood amino nitrogen of children with cystic fibrosis of the pancreas following ingestion of the protein hydrolysates was very similar to the increases observed in normal children following ingestion of the intact proteins.

Simultaneous administration of an enteric coated pancreatin preparation along with intact casein to children with cystic fibrosis caused a greater increase in the blood amino nitrogen concentration than when casein was given alone.

Lavik et al. (80) studied protein digestion and absorption in children with or without cystic fibrosis of the pancreas by use of  $I^{131}$ -labeled proteins. They compared the results of five children between the ages of 1.6 and 4.5 years with cystic fibrosis of the pancreas to ten children between the ages of 2 and 6 years with no disease of the pancreas. The patients consumed a test meal containing  $I^{131}$ -labeled casein and the urine and feces were collected for 72 hours after the test meal was ingested. From the results they concluded that less than 6.0 percent of the ingested isotope was excreted in the feces of the normal patients in the 72 hour period, whereas an average of 22.5 percent was excreted by the patients with cystic fibrosis of the pancreas. Although the percent of the isotope excreted in the urine of the total amount ingested was lower for the children with cystic fibrosis of the pancreas than the normal patients, the difference was not nearly so great as was encountered in the fecal excretion rates. Inclusion of pancreatin in the test meal of the cystic fibrosis patients reduced the amount of the isotope excreted in the feces by 50 percent. In preliminary studies using the dog, they established that less than 12 percent of the  $I^{131}$  is liberated from the protein as inorganic iodide, with the greatest liberation rate occurring in the duodenum, and further that the majority of the  $I^{131}$  is attached to tyrosine as the moniodotyrosine or diiodotyrosine moities upon hydrolysis of the

$I^{131}$ -labeled casein. They also reported that the  $I^{131}$  activity of the blood of depancreatized dogs during the first 3 hours after ingestion of an  $I^{131}$ -labeled casein test meal rose at a slower rate to a value approximately one-half of that for normal dogs.

Chinn et al. (17) applied identical procedures as that reported immediately above (80) to the study of protein digestion and absorption of five adult patients with chronic pancreatic insufficiency and eleven patients with no gastrointestinal diseases. The results of this study strongly confirm the results of the study reported by Lavik et al. (80). The data indicate that the fecal excretion pattern of the  $I^{131}$  is of more reliable diagnostic aid than the urine excretion pattern.

The evidence presented above indicates that the digestibility of protein and dry matter of a soybean diet by the young pig is low at birth and increases with age so that at the age of 7 to 8 weeks the digestibility approaches that obtained with dried skim milk basal diet. The utilization of protein and dry matter by baby pigs fed a dried skim milk diet improves slightly with age. This evidence leaves little doubt that the efficiency of utilization of the diet by the pig improves appreciably from birth to 8 weeks of age. Previous investigators generally favor the concept that this improvement in diet utilization is due to insufficient elaboration of the digestive enzymes by the pig shortly after birth and that the exaggerated improvement in the case of soybean protein digestibility as compared to milk protein digestibility is due to the complexity of plant proteins. The evidence presented above shows that supplementation of the diets with a variety of proteolytic and amylolytic enzymes or

dietary hydrolysates seldom improves and occasionally reduces the digestibility of the diet in spite of improving the performance of baby pigs fed the supplemented diets. The concentrations of enzymes in the tissues of the digestive glands or organs of baby pigs at various ages reveal characteristic changes in the various enzymes elaborated, however the total weight of the organs was also found to be affected by the dietary regimen. In those cases where total glandular enzyme activities were calculated, the interpretation was considerably different than the interpretation based on the enzyme activity per unit of tissue. In addition, the enzyme activity of the ingesta of the small intestine and stomach do not mutually support the interpretations indicated by the enzyme activity of the tissues of the small intestine and stomach. In one specific case the protease activity of the pancreatic tissue of baby pigs was found to be lower for weaned pigs fed a dry diet than for suckling pigs, but the weaned pigs had considerably more ingesta in the small intestine and considerably more protease activity per gram of ingesta and therefore excessively more total protease activity in the small intestine than the suckling pigs. Until a reliable correlation between the enzyme activity of glandular tissue and the enzyme activity liberated into the lumen of the digestive canal by the gland can be demonstrated, changes in the enzyme concentration of tissue and ingesta cannot be considered unequivocal bases for the evaluation of digestive function of the gland under question. This indicates the need for evaluating the actual output of the digestive secretions of the digestive glands. In addition in vitro studies with the secretions obtained would be of value. Bondi and Birk

(10) have demonstrated marked differences in the pancreatic digestion (in vitro) of plant and animal proteins.

### Exocrine Pancreatic Function and Properties of Pancreatic Secretion

#### Morphology and development of the pancreas

Patten (111) stated that the pancreas is derived from two separate primordia, giving rise to the dorsal and ventral pancreatic buds, which later become fused. The glandular tissue is formed by budding and re-budding of cords of cells derived from the primordia. The terminal parts of these cords of cells eventually become the pancreatic acini, while the proximal portions form the ducts. In the adult pig the duct derived from the ventral pancreas, duct of Wirsung, usually loses its continuity with the gut and the duct of Santorini persists. Sisson and Grossman (123) stated that in the mature pig "the pancreas extends across the dorsal wall of the abdominal cavity behind the stomach. It is triradiate or triangular. The right extremity is attached to the first curve of the duodenum, and here the duct passes to the bowel. The left extremity is related to the left extremity of the stomach, the dorsal end of the spleen, and the anterior pole of the left kidney. The pancreatic duct passes from the right extremity directly through the duodenal wall, opening about four or five inches from the pylorus. The interlobular tissue usually contains a good deal of fat." It is probably significant that a papilla of Vater characteristic of the dog and human was not specifically

mentioned in this description of the pancreas of the pig. The pancreas of the pig receives its blood supply primarily from branches derived from the coeliac artery and anterior mesenteric artery.

According to Thomas (129) the pancreas receives an abundant nerve supply from both the vagi and splanchnic nerves.

### Experimental methods

Investigators studying the properties and characteristics of the pancreatic secretion or juice per se under various experimental conditions are limited to those methods involving direct collection of the pancreatic juice. Thomas (129) described the various experimental methods for collection of pancreatic juice. More recently the same author published a separate report (130) in which methods for the collection of pancreatic juice are summarized. He stated that, ideally, pancreatic juice should be collected from an unanesthetized animal in good health with the nerve and blood supply of the pancreas intact, and that it should be possible to collect the total secretions over an extended period of time, and to collect the juice secreted in response to each of the known pancreatic stimuli as well as that secreted in response to meals. He added that this ideal method has not been achieved, nor is it likely to be since some of the conditions are mutually exclusive, but that certain of the methods offer a reasonable approximation to the ideal condition. He also reported that pancreatic fistulae can be grouped into three general classes, temporary, semipermanent, and permanent. He further discussed the various modifications of each type used by investigators and pointed out the

advantages and disadvantages of each, therefore it seems unnecessary to include a similar evaluation of these methods here, except to include a very brief description of the three classes of pancreatic fistulae. In accordance with the classification of Thomas (130) a temporary pancreatic fistula involves cannulation of the pancreatic duct in acute experiments. The primary limitations of this type of fistula are those imposed by the anesthetic and other physiologic involvements encountered in an acutely operated animal. It is most useful in studies of the effects of humoral agents on the exocrine pancreas. The semipermanent pancreatic fistula differs from the temporary fistula in that the animal survives the operative procedures and is thus subject to postoperative observation. It differs from the permanent fistula because the cannula eventually comes out of the duct or the fistulous tract created by other means becomes obstructed. The first pancreatic fistula to be reported in the literature was of this type. The permanent pancreatic fistula is one which provides for a continuous flow of pancreatic juice during the remainder of the animal's life. It is prepared by a variety of techniques which require that a section of the duodenum containing the pancreatic duct be transplanted to the skin or subcutaneous tissues of the abdominal wall after repair of the duodenal wound, or by securing a permanent cannula into an isolated segment of duodenum into which the pancreatic juice is secreted. Pavlov (112) prepared the first permanent fistula in his classical experiments. The most serious limitations of this type of fistula are; severe irritation of the skin in the area of the fistula due to activation of the trypsinogen of the pancreatic juice to trypsin by the small collar of

duodenum, high mortality rate, and interruption of the blood and nerve supply to the pancreas. Some of these limitations have been essentially annihilated by modifications.

Since pancreatic fistulae of the permanent type were used in this study a more detailed review of their limitations is expedient. Pavlov (112) discussed some of the limitations he encountered in using permanent pancreatic fistulae on mature dogs. He stated that pancreatic juice secreted via a fistula immediately after establishment of the fistula is small in quantity and of abnormal composition and therefore experimental observations on the physiological influences on the secretion rate or composition of the juice are of limited value. In his opinion this is because the pancreas is a very sensitive organ, and suffers such severe disturbance from the unavoidable conditions of the surgical operation that in the majority of instances not a trace of normal secretory action remains; therefore it is essential that the animal recover from the operation before valid physiological experiments are conducted. He further stated that the pancreas gland remains normal for a considerable period after recovery of the operation, but the abdominal wall in the area of the transplanted duct is severely irritated by digestive ferments. He concluded that there were no apparent digestive disturbances reflected by fecal properties or vomiting associated with the fistula. He found that after 3 or 4 weeks the animals usually go into convulsions, refuse food, and die. He established that death was not due to starvation or infection by exclusion of meat from the diet and addition of sodium bicarbonate which maintained the animals in good health for months and from this he

concluded that death was due to the loss of essential components in the pancreatic juice.

Later, Gamble and McIver (37) specifically studied body fluid changes due to continued loss of pancreatic juice via a fistula. They reported that the volume and composition of the blood plasma remained very close to normal during the first two-thirds of the survival period, the losses of water, sodium, and chloride ion being replaced at the expense of interstitial fluids. Body weight loss began immediately after establishment of the fistula which indicated a reduction of the volume of interstitial fluids. Ultimately reduction of plasma volume began, and as it progressed, serious symptoms developed and death followed shortly thereafter, unless water, sodium, and chloride ions were abundantly replaced. There was a greater loss of sodium than chloride ions in the pancreatic juice associated with a reduction of the bicarbonate ion concentration in the plasma. In their opinion death may be simply and reasonably explained as the result of progressive impairment of the function of the blood by the physical changes, dehydration and acidosis produced in the plasma by the continued loss of sodium, chloride and bicarbonate ions in the pancreatic juice.

Thomas (129) stated that pancreatic fistulae can be partial or complete, depending on whether the total secretion from the entire gland is forced to be secreted via the fistula, or if some of the secretion can escape into the intestine. Partial pancreatic fistulae are easily established in the dog by fistulation of the duct of Wirsung, leaving the duct of Santorini intact. Thomas (129) summarized the published opinions

and results of many investigators and he concluded that no pancreatic fistula yet devised is entirely satisfactory. He stated that complete fistulae always result in death of the animal unless the fluid and base lost through the pancreas is regularly replaced; the survival time was short in any case. He also expressed the opinion that partial fistulae yield an uncertain fraction of the pancreatic juice and are therefore quantitatively unsatisfactory. He emphasized that digestion and absorption are disturbed by the loss of pancreatic juice particularly in the case of the complete fistula and also that the pancreas tended to hypersecrete when fistulated.

Pfeffer and Hinton (115) have obtained evidence that the exocrine secretion of the pancreas is inhibited during periods of stress due to the sympatheticomimetic effects of adrenaline and adrenocorticotrophic hormone.

Thomas (130) outlined a method for the collection of pancreatic juice without a pancreatic fistula, thus no physiological or anatomical stress need be imposed on the gland or its blood and nerve supply. The method allows intermittent collections of pancreatic juice at will by inserting a cannula into the opening of the pancreatic duct made visible by establishment of a permanent duodenal fistula so placed as to be directly opposite the opening of the major pancreatic duct. The main disadvantage of this method is that the animal must be observed constantly during the collection of the pancreatic juice and the orifice of the duct must be clearly visible.

Rous and McMaster (119) describe a method for sterile drainage of the common duct.

Investigators studying exocrine pancreatic function rather than pancreatic juice per se have a larger selection of methods available to them.

Popper and Necheles (117) very recently, 1959, summarized the various methods for evaluation of pancreatic function under clinical conditions. The methods listed by them were categorized into methods useful for the diagnosis of acute pancreatitis, pancreatic duct obstruction, and insufficient exocrine pancreatic secretion. The latter two have a direct bearing in this study and therefore will be reviewed in more detail. To test for pancreatic duct obstruction, they suggested analyzing the serum of the subject for activity of the enzymes secreted by the pancreas and indicated that trypsin is probably the best. This test is based on the theory that the pancreatic enzymes diffuse out of the gland proper into the interstitial tissues and subsequently appear in the blood if resistance is encountered in the duct. An alternative method is to evaluate the antithrombin titer of the blood which is indirectly related to the trypsin concentration. Also analysis of duodenal fluid for enzymes of pancreatic origin was suggested as a diagnostic aid reflecting a possible blockage of the pancreatic duct.

A larger selection of methods was suggested for evaluating the adequacy or inadequacy of the exocrine pancreatic secretion. Serum enzyme analysis was recognized as a diagnostic aid in this regard but of very limited usefulness. Analysis of fecal excreta for specific digestive residues and starch tolerance tests involving measurement of the blood glucose concentration after a starch test meal and comparing these results

to values obtained after a glucose test meal were mentioned. Also measurement of plasma glycine after a protein test meal, or the  $I^{131}$  concentration of blood after ingestion of the isotopically labeled protein, fat, or fatty acid has met with success.

A method which has proven quite useful for clinical use is the hormonal secretin test. Secretin (a hormone which is discussed in more detail below), when injected intravenously causes the pancreas of normal patients to secrete a large quantity of pancreatic juice characterized by a high concentration of bicarbonate and a low concentration of enzymes. Thomas (131) discussed the results of early attempts to use secretin to test pancreatic function.

Dreiling (29) expressed the opinion that the secretin pancreatic function test has good usefulness for clinical diagnosis of pancreatitis and for demonstrating a pancreatic insufficiency in cases of gastrointestinal disorders. The method as used by him required that a double lumen gastroduodenal tube be positioned in the duodenal loop of a fasted patient by fluoroscopic observation. The gastric and duodenal contents were then aspirated for a 20 minute period. At this time 1.0 clinical unit of secretin was injected intravenously per kilogram body weight and the gastric and duodenal contents were aspirated for an additional 80 minutes and the specimens separated into four 20-minute periods. The volume, bicarbonate and amylase content, and the icterus index were determined and the total output calculated. The results were interpreted by comparing them to data accumulated from normal patients.

Sun and Shay (127) investigated the usefulness of a combined study of serum enzymes and duodenal contents after secretin administration in the diagnosis of diseases of the pancreas. They administered 80 units of secretin suspended in 16 milliliters of isotonic saline to the patients by intravenous injection. They found that injection of isotonic saline alone as a control increased serum amylase and lipase by 40 and 250 percent respectively. Secretin did not cause the serum enzymes of normal patients to rise more than the isotonic saline alone and it gave inconsistent increases of serum enzymes in patients with pancreatitis. They also reported that some patients with pancreatitis secreted similar quantities of pancreatic juice into the duodenum after secretin administration as normal patients, while the serum enzymes increased significantly more than in normal patients. They are of the opinion that serum lipase is more likely to increase after secretin administration than serum amylase and that measurement both of serum enzymes and of pancreatic secretion into the duodenum is more likely to lead to a correct diagnosis of a pancreatic disorder.

A few years later the same authors, Sun and Shay (128), reported the results of a very similar investigation as the one described above, except that pancreozymin was administered with secretin. Twenty-five normal patients and 27 patients with pancreatic diseases were fasted for 14 hours, and the duodenal contents aspirated for 20 minutes. Then 90 units of pancreozymin (5 units per milliliter of isotonic saline) were injected intravenously at a rate of one milliliter per 15 seconds and the duodenal contents were aspirated the following 10 minutes. This was followed

immediately by intravenous injection of 80 units secretin (5 units per milliliter of isotonic saline) at a rate of two milliliters per 15 seconds and the duodenal contents were aspirated for the following 60 minutes for four periods of 10, 10, 20, and 20 minutes. Blood samples were collected at intervals of 1, 2, and 4 hours after secretin administration. They concluded from the results that the combination of pancreozymin and secretin increased the reliability of the test as compared to secretin alone, and that the measurement of the enzyme activity of both the blood and duodenal juice increased the reliability of the test since some patients with pancreatic disease gave normal results for one measurement but abnormal for the other.

Lavik et al. (80) and Chinn et al. (17) used  $I^{131}$ -labeled casein in a test meal and measured the rates and pattern of excretion of the isotope in the feces and urine and also observed the changes in blood content of the isotope. The method yielded marked differences between normal patients and patients with pancreatic disorders. Shingleton et al. (121) used  $I^{131}$ -labeled fats in studies of pancreatic disorders and the results were in good agreement with those obtained with  $I^{131}$ -labeled protein (80), (17).

Another experimental method for the study of exocrine pancreatic function is to perform a partial or complete pancreatectomy and observe the effects on nutrient digestibility. A similar but more popular method to exclude pancreatic digestion is that of pancreatic duct ligation. Thomas (129) stated that modern investigators have generally found a pronounced reduction in digestion and absorption following pancreatectomy

or ligation of the pancreatic ducts. Since pancreatic duct ligation was among the major experimental techniques used in this study, it is pertinent that the results obtained from other investigations, in which pancreatic duct ligation was used, be reviewed here.

Thomas (129) discussed and summarized the results of several investigations reported prior to 1940 in which pancreatic duct ligation was practiced. He states that the results of some of the early investigations were conflicting and the proposals brought forth to explain these conflicts included the possibility that after ligation of the pancreatic ducts, pancreatic enzymes were absorbed by the blood and subsequently secreted by other glands or that sinuses were formed which reestablished communication between the pancreas and the intestine.

Pavlov (112) stated that ligation and division of the pancreatic ducts of dogs was found to be a perfectly harmless operation. Wang et al. (141) found that ligation of the pancreatic ducts of rabbits did not cause pancreatitis, but did cause atrophy of the acinar tissue and dilation of the ducts.

Gayda (38) reported that the amylase content of saliva remained unchanged in the rabbit after ligation of the pancreatic duct, whereas the amylase content of the blood increased initially and later returned to normal. He stated that not all the amylase of the blood originates from the pancreas and that the islets of Langerhans are not affected by the ligation of the duct. Maj and Bonora (98) also observed the salivary amylase concentration following partial or complete ligation of the pancreatic ducts of three dogs maintained on a diet of bread and meat.

They found no change in the salivary amylase concentration immediately after the operation, but subsequently the concentration began to rise and reached a maximum in 30 to 60 days; it remained elevated with a slight tendency to decline until the experiment was terminated 2 to 3 months after the operation. During the time when the amylase value of the saliva was high, that of the blood remained normal. It was suggested that the amylase of the pancreas was possibly stored in the salivary glands and later released in the saliva or more probably that the activity of the salivary glands was increased in response to some stimulus arising in the pancreas.

Popper and Sorter (118) ligated and sectioned the pancreatic ducts of four dogs. Two died from pancreatitis shortly after the operation. Of the two dogs that survived, one lost weight continually, the other retained its original weight for 9 months post-ligation when it suddenly lost weight. Both of the latter two dogs died 12 months after the ducts were ligated. After death the pancreas was found to be a small cordlike structure which did not resemble pancreas at all. The tissue contained an abundance of connective tissue with many nerve fibers, blood vessels, and islets of Langerhans, but only a few isolated nests of acinar cells. The serum amylase of one dog increased 24 hours post-ligation but it returned to normal 11 days later and remained normal up to the time of death. Serum lipase for the same dog was not found to be abnormal, however it was not determined the first 11 days post-ligation. The serum amylase of the other dog was high at 1 day, 6 days, 6 weeks, and 11 weeks post-ligation after which it dropped and remained below the pre-ligation levels until

death. Serum lipase was found to be normal throughout the post-ligation survival period of the dog except for one marked rise 6 weeks after the ducts were ligated. They reported that the serum enzymes would not respond to Mecholy-esterine after 21 weeks post-ligation which suggested to them that no functional pancreatic acinar tissue remained. They expressed the opinion that the secondary and tertiary rises in serum enzymes were due to inflammatory processes.

Nothman et al. (110) reported that serum lipase increased after ligation of the pancreatic ducts. In a subsequent operation on the same animals a total pancreatectomy was performed, after which there was an immediate drop of the serum lipase to almost zero values in 5 days which was followed by a recurrence of serum lipase 2 to 3 weeks after the pancreatectomy. They concluded that the increase in serum lipase after pancreatic duct ligation was largely of pancreatic origin, but that serum lipase does originate from extrapancreatic sites as well as from the pancreas.

Gibbs and Ivy (39) have studied the histologic changes that occur in pancreatic tissue and the serum amylase levels during the first 24 hours after ligation of the pancreatic ducts of dogs maintained under sodium pentobarbital anesthetic. The dogs were fasted for 20 hours before commencement of the experiments. The pancreas was not intentionally stimulated in their experiments. Three control dogs were observed by exposing the pancreatic duct without ligating it. They found very little change or variation in serum amylase concentration of the control dogs and there was no edema of the gland or histological variations after 24 hours.

On the contrary, three dogs with the pancreatic ducts ligated demonstrated a persistent and marked rise in serum amylase throughout the experimental period. The pancreas appeared edematous 5 to 7 hours after the ducts were ligated. Histologically the interlobular septa was spreading and the spaces were infiltrated with leukocytic cells. There was no apparent alteration of the acinar cells. In addition the pancreatic ducts of six dogs were cannulated and exposed to a constant pressure of 30 centimeters of water. Three dogs died in 15 hours, two were killed after 12 hours, and the other lived the full 24 hours. They found that the rise in serum amylase was more rapid and of a greater magnitude than was observed with ligated pancreatic ducts. Also edema appeared earlier and was more severe. Infiltration of the pancreatic tissue by inflammatory cells was more intense; leukocytic cells were identified in the interlobular spaces, among the acini cells, and occasionally in the ducts. There was some apparent acinar cell degeneration.

Karvinen et al. (69) studied the effects of pancreatic duct ligation on triglyceride digestion and absorption of 12 rats with the bile duct transplanted to the jejunum. Also 12 sham operated control rats were prepared by transplanting both the pancreatic duct and the bile duct to the jejunum. The rats were maintained on basal diet supplemented with pancreatin and corn oil for a few days before the actual experiment during which time the rats all continued to gain weight. During the experimental period fat balance data were collected from the rats consuming a specific diet, each containing one of five fats, tripalmitin, trielaidin, triolein, tallow, or corn oil, at the rate of 8.0 percent of the total diet. They found that

fat and soap excretion in the feces was always higher for the rats with pancreatic ducts ligated than the sham operated control rats and that the relative difference between the five fats was more closely related to their respective melting points than their degree of saturation. The same authors in a separate report (70) involving rats prepared in the same way as was described before (69) found that the exclusion of pancreatic juice significantly reduced the utilization of triolein and tripalmitin. The degree of reduction in utilization of the two fats was of similar magnitude. The absence of pancreatic juice, however, did not reduce the utilization of oleic or palmitic acid. From this they concluded that pancreatic juice improved the utilization of fats of dissimilar degrees of saturation in a similar way, but did not improve the absorption of the respective free fatty acids. They also reported that most of the triolein recovered in the feces of rats with the pancreatic ducts ligated had undergone hydrolysis which they suggest could have been accomplished by intestinal esterases. In summary they listed four ways in which pancreatic juice can improve fat utilization; hydrolysis of fat, formation of soap due to the bicarbonate ion, maintenance of a favorable pH for fat hydrolysis, and maintenance of a favorable pH for fat and fatty acid absorption.

Still another experimental approach to the study of specific processes in pancreatic tissue is that of the analysis of the tissue itself either by histological, chemical, or histochemical techniques. Methods falling within this class were used by; Daly and Mirsky (25); Junqueira et al. (67,68); Hokin (56,57,60); Hokin and Hokin (58,59); Hansson (49); Kitts et al. (74); Lewis (82); Lewis et al. (85); Magee (93,94,96,97);

Fernandes et al. (35) and others.

Collection and analysis of duodenal contents and analysis of blood for specific dietary components following a test meal or for pancreatic enzymes have already been mentioned in this section of the review because they were used in conjunction with other pancreatic function tests.

#### Properties and composition of pancreatic juice

The general characteristic properties of pancreatic juice will be reviewed here. Factors which affect these properties will be discussed in another section (see Factors Regulating the Exocrine Secretion of the Pancreas).

Thomas (129), who has summarized the results of many investigations into the properties and composition of pancreatic juice, stated that pancreatic juice is generally a colorless, odorless, alkaline fluid of low viscosity, tasting strongly of sodium bicarbonate, however, a few exceptional specimens having a high concentration of enzymes may be viscous and even jelly at low temperatures. He cited a report on 60 specimens of pancreatic juice from healthy dogs, the pH values remained in the narrow range of pH 8.0 to pH 8.3. The specific gravity of the pancreatic juice of dogs, measured gravimetrically at room temperature, fell between 1.007 and 1.042 depending largely on the amount of protein contained. The osmotic activity of pancreatic juice was the same as that of the blood when the pancreatic juice and blood were collected simultaneously from the same animal. Experimental alteration of the osmotic activity of the blood resulted in a corresponding change in the pancreatic juice. Regarding the composition of pancreatic juice, he stated that the distinguishing

chemical characteristic of pancreatic juice is its high bicarbonate content, and that it is a well established fact that the bicarbonate and chloride concentrations vary in a reciprocal manner so that the sum of the two, expressed in milliequivalents, is constant and nearly the same as the total base of the blood plasma. In addition pancreatic juice contains a small amount of phosphate, the concentration being less than is found in blood plasma. Sodium and potassium are found in pancreatic juice at concentrations equivalent to those of blood plasma, however, calcium is also present in pancreatic juice, but at a concentration less than that of blood plasma.

Dreiling and Janowitz (30) reported that zinc and sulfate are contained in pancreatic juice in addition to the six inorganic ions already discussed.

Thomas (129) stated that the protein content of pancreatic juice of the dog varies between 0.1 and 10.0 percent in a complicated but orderly manner with the conditions governing secretion. Values obtained from specimens of human pancreatic juice indicate that the protein content is nearer the lower extreme found for the dog. He also cited experimental evidence which shows that electrophoretic fractionation of the proteins of pancreatic juice in sodium bicarbonate buffer at pH 8.2 yields 4 and frequently 5 separate components. A sixth component was frequently found if a sodium diethylbarbiturate buffer at pH 8.6 was used. The relative concentrations of the several fractions were fairly constant regardless of the concentration of the total protein. He speculated that the four major fractions separable by electrophoresis may correspond to the enzymes known

to be present in pancreatic juice.

In review of the enzymes contained in pancreatic juice, Thomas (129) stated that pancreatic juice promotes the hydrolysis of all three classes of foodstuffs and is in fact the most active and versatile of all the digestive secretions. Pancreatic juice owes its digestive activity to the presence of proteolytic, amylolytic, and lipolytic enzymes and to the inorganic constituents which provide a favorable medium for their activity. The proteolytic activity of the juice is due to the presence of trypsinogen and chymotrypsinogen, which are inactive as such, but are activated upon entrance into the duodenum by enterokinase contained in the duodenal secretion to trypsin and chymotrypsin respectively.

Kuhn (76) was the first to designate the proteolytic agent in pancreatic juice as trypsin. Investigations, too numerous to cite here, led to characterization and crystallization of trypsin by Northrop and Kunitz (108), of trypsinogen by Kunitz and Northrop (78), of chymotrypsin and chymotrypsinogen by Kunitz and Northrop (77).

The term chymotrypsin is a term which is applied to at least five known members of a closely related group of enzymic proteins, all of which are derived from chymotrypsinogen and have the same proteolytic activity. Chymotrypsinogen has a molecular weight of about 25,000 (36, p. 692).

According to Northrop, Kunitz, and Herriott (109, p. 105) chymotrypsinogen is a protein with a molecular weight of about 36,700 and after repeated crystallization it can be activated only by the action of trypsin whereas crude chymotrypsinogen solutions can be activated only by enterokinase (109, p. 106). Chymotrypsin activity is optimal at about pH 8,

resembling trypsin very closely (109, p. 118). Trypsin and chymotrypsin are the enzymes principally responsible for the protease activity of pancreatic juice. Neither alone digests protein very far, however the two together cause hydrolysis to proceed to the polypeptide stage (109, p. 97).

Northrop et al. (109, p. 140) state that trypsin has a molecular weight of about 34,000. Trypsin is the enzymatically active product of enterokinase action on the inactive precursor, trypsinogen. Enterokinase was discovered by Schepowalnikow in Pavlov's laboratory in 1899 (109, p. 127). Trypsin can also be formed from trypsinogen by the autocatalytic action of trypsin itself (109, p. 126). It is also stated that trypsin is rapidly digested by pepsin in an acid medium, and the decrease in protein nitrogen under such conditions is proportional to the decrease in activity (109, p. 138). Thomas (129) stated that spontaneous activation of trypsinogen in the living pancreas is suppressed by the presence of an inhibitor. An enzyme possessing carboxypeptidase activity has been obtained in crystalline form from pancreatic tissue by Anson (5). It occurs as an inactive protein, procarboxypeptidase, and like chymotrypsin it is activated by trypsin.

Pancreatic amylase was crystallized by Caldwell et al. (11) in 1931 and the amino acid composition of the crystalline enzyme was reported by Caldwell et al. (12) in 1954.

Pancreatic lipase was purified only recently for the first time. Marchis et al. (99) have outlined a method for the purification of hog pancreatic lipase in 1959.

Harper (51) stated that pancreatic juice contains cholesterol esterase, ribonuclease, deoxyribonuclease, and collagenase. Grant and Robbins (40) have recently outlined methods for partial purification and characterization of a new pancreatic proteinase, pankrin. More recently, 1961, Kessler, et al. (72) obtained evidence that pancreatic juice contains lipoprotein lipase activity.

#### Functions of the exocrine secretion of the pancreas

Thomas (129) stated that the most important function of pancreatic juice is to aid in digestion and absorption of the major foodstuffs. Harper (51) stated that the high alkaline content of pancreatic juice along with biliary secretions neutralizes the acid of the gastric chyme and changes the pH of the material to the alkaline side which is necessary for the activity of the enzymes contained in pancreatic and intestinal juice.

The variety of digestive enzymes contained in pancreatic juice (reviewed immediately above) corroborates the prominent digestive role of this secretion. Guyton (47, p. 735) has stated that it would be almost possible for a person to live if he secreted no other digestive juice besides that of the pancreas. The digestive disturbances caused by excluding pancreatic juice have been discussed elsewhere in this review in considerable detail and will not be repeated here.

### Factors Regulating the Exocrine Secretion of the Pancreas

In 1950, Thomas (129) and again in 1959 Harper (50) summarized the experimental evidence concerning the physiological mechanisms which control the exocrine secretion of the pancreas. Therefore this section will be reviewed in a general manner, except that the recent literature reflecting dietary influences on the pancreas will be treated in greater detail since it is of prominent importance in this study.

Harper (50) stated that nervous and hormonal control of the secretion of the pancreas were indicated in published reports at the turn of the nineteenth century, a time coincident with the classical experiments of Pavlov (112) and of Bayliss and Starling (9).

Thomas (129) pointed out that the results obtained with various types of pancreatic fistula differed and therefore evidence of the "normal" is dependent upon selection of the proper fistula. The results of early investigators suggested that the secretion of herbivores was continuous whereas that of carnivores was apparent only after proper stimulation. It is accepted that the secretion of the rabbit, white mouse, white rat, guinea pig, and frog are continuous as is also the general rule in the case of the human patient.

Pavlov (112) produced conclusive evidence that the vagus nerve does carry stimulatory fibers to the pancreas by stimulation of the peripheral end of the nerve sectioned four days earlier. He also provided evidence that the vagus also carries inhibitory fibers to the pancreas.

Crittenden and Ivy (20) studied the nervous control of the pancreas secretion in the dog after complete removal of the small intestine to eliminate the secretin mechanism. They concluded; that stimulation of the dorsal trunk of the vagus causes a marked increase in enzyme secretion but has negligible effect on the volume of secretion, that the trophic fibers of the vagus are all contained in the dorsal trunk, that the splanchnic nerves contain fibers which inhibit the acinar cells and that stimulation of the central end of the vagus or sympathetic nerves of the pancreas has no effect on the pancreas.

Thomas (129) acknowledged that the pancreas is stimulated by the act of eating in two separate phases, cephalic and intestinal. Cephalic stimulation is that which causes the pancreas to secrete before or during the time food is being received into the gastrointestinal tract but has not yet reached the stomach. This was proved by Pavlov (112) using dogs with an esophagostomy so that the food was masticated and returned to the exterior upon deglutition. He also demonstrated cephalic stimulation of the pancreas using dogs prepared with gastric fistulae so that the acid produced by the stomach by cephalic stimulation was prevented from entering the duodenum. In this case the pancreas response was apparent before the gastric response. Alphin and Lin (1,2) have reported that stimulation of the pancreas by feeding rats prepared with esophagostomies was greatly reduced by draining the gastric contents via a fistula as compared to rats with no gastric fistulae. On the other hand, gastric feeding induced an increase in the volume and amylase output of the gland.

Evidence obtained by Anderson et al. (4) and Sinclair (122) suggests a lack of cephalic stimulation for the human; however each report was based on a single patient.

Thomas (129) indicated that foods in the stomach apparently do not stimulate the pancreas, but upon passage into the duodenum they become very effective stimuli. The latter is referred to as the "intestinal phase" of pancreatic secretion. He added that all the major constituents of gastric chyme influence the secretory activity of the pancreas in one way or another.

Pavlov (112) stated that gastric acid does not effectively stimulate the pancreas until it enters the duodenum. The quantity of acid was reported to influence the inorganic constituents of pancreatic juice but not the organic fraction; the influence was thought to be mediated via nervous reflexes.

Bayliss and Starling (9) were the first to propose that a hormone, which they named secretin, was liberated from the intestinal mucosa by the action of acid in the small intestine which was then carried by the blood to the pancreas where it acted as a secretory stimulus. They also prepared an extract of the small intestine which produced the same effects on the pancreas when injected into the blood of an animal as was observed by placing acid into the jejunum.

Harper (50) reported that by 1920 the existence of a humoral phase of pancreatic secretion had been clearly proven by Farrell and Ivy (34) who demonstrated that a pancreas transplanted into the mammary gland of a lactating bitch could be stimulated to secrete by consumption of food or

acid solution.

Harper (51, p. 156) reported that secretin as prepared by Bayliss and Starling has been separated into five separate active hormonal components, namely secretin, pancreozymin, hepatocrinin, cholecystokinin, and enterocrinin. Of these, only secretin and pancreozymin play a significant role in the physiological control of the pancreas.

Mellanby (101) postulated that the pancreas was under dual control, nervous and humoral, and that the nervous system reflexly controlled enzyme secretion, while secretin controlled the volume and bicarbonate output.

Harper and Vass (53) observed that the passage of the gastric contents into the duodenum of cats, with all the extrinsic nerves of the duodenum sectioned, caused a marked increase in the enzyme output of the gland. This led Harper and Raper (52) to investigate among other possibilities that an enzyme-stimulating hormone elicited the pancreatic enzyme response to gastric chyme in the duodenum. They were successful in isolating a substance from the small intestine of the pig, dog, and cat which on intravenous injection caused an increase in the enzyme secretion of the pancreas but did not produce the characteristic secretin effect. They named this substance pancreozymin.

Harper (50) gave evidence that pancreozymin as prepared by them (52) contains other physiologically active components which have successfully been separated from the pancreozymin. He also cited evidence that substances have been extracted in the pyloric antrum of the stomach of the pig which elicit characteristic secretin and pancreozymin effects on

the pancreas. He stated that "unless it can be shown that there is in the pig a gastric phase of pancreatic secretion which is absent in the ordinary laboratory animals, no physiological significance can be attributed to the demonstration of pancreatic excitants in the antral mucosa".

According to the evidence summarized by Thomas (129) secretin may be a polypeptide with a molecular weight of approximately 5000, although some contend that it is not a polypeptide. It is digested by aminopeptidase but not by carboxypeptidase.

Regarding the influence of the diet consumed by an animal on the secretion of the pancreatic juice, Pavlov (112) was among the first to describe the marked but complex influence that the diet exerts on the digestive glands. In his lectures he reflected remarkable confidence in the precision with which the glands can adjust the secretion volume to the quantity of foods consumed. He acknowledged, however, that the effect of the diet on the glandular secretion is dependent upon the physical and chemical properties of each of the foodstuffs composing the diet.

The following quotation from Pavlov (112) pertaining to the dietary influence on the composition of pancreatic juice is referred to as Pavlov's "purposive adaptation" theory: "if in the feeding of animals, the kind of food be altered, and the new diet maintained for a length of time, it is found that the ferment content of the juice became from day to day more and more adapted to the requirements of the food". He qualified this statement by adding that these purposive changes can be shifted in either direction by appropriate diet changes proving it was not a

spontaneous and unavoidable event.

Thomas (129) stated that Pavlov's theory was based on the determined enzyme activities of pancreatic juice which was not activated. Since Pavlov's theory was published, enterokinase was discovered, as was the activating effect of bile salts on lipase. He added that Babkin repeated the experiments leading to Pavlov's theory with proper activation of the enzymes and found that although the total enzyme activity varied with different meals, the individual enzymes were secreted parallel to each other; thus the "parallelism theory" of Babkin.

More recently, 1956-58, Guth et al. (45,46) have concluded from their experiments with dogs that neither Pavlov's "purposive adaptation" theory, or Babkin's "parallelism" theory could be verified. By use of statistical procedures they found that the variation of the ratios of enzyme activities to protein nitrogen of pancreatic juice and the variation of the total activities of the three enzymes secreted in 3 hours after a variety of test meals was no greater than the daily variations observed using the same test meal.

Grossman et al. (43) found that the enzyme ratio of the extract of pancreatic tissue of dogs is the same as the ratio of the pancreatic juice secreted in response to secretin and concluded that dietary influences could best be studied with pancreatic tissue. The results they obtained using pancreatic tissue of rats indicate that a high carbohydrate diet increased the amylase and decreased the trypsin, a high protein diet increased the protease, a high fat diet had no marked effect on any of the enzymes whereas a high fat, low protein diet depressed all three enzymes.

Muto (105) reported that the trypsin content of the pancreatic juice of dogs increased after a protein meal and that the amylase increased after a carbohydrate meal.

Grossman (41) investigated the possibility that the diet could influence the secretion of the pancreas directly by a humoral mechanism in which the dietary products absorbed into the blood stream would serve as the humoral agent. Amylase, lipase, and protease activities of pancreatic tissue were evaluated on four groups of rats. One group received a balanced diet containing casein and corn starch, a second group received the same diet except that the corn starch was replaced by an equivalent amount of dextrose, in the third group the casein was replaced by an equivalent amount of casein hydrolysate, and the fourth group received the same diet as the first plus daily injections of insulin. He found that dextrose increased the amylase and lipase activities of the tissues, casein hydrolysate decreased the protease activity, and insulin decreased the amylase activity. He concluded that the results suggest a humoral mechanism by which the diet could stimulate the pancreas.

After the discovery of pancreozymin, Wang and Grossman (140) set out to investigate the effects of various digestive products in the small intestine on the secretion of the transplanted pancreas of dogs. They found that water was a weak stimulant whereas saline was not, that hydrochloric acid elicited a powerful secretin effect (volume response) and a mild pancreozymin effect (enzyme response), that peptones and amino acids powerfully stimulated enzyme secretion and volume of secretion, soap stimulated the gland in a similar manner as peptones and amino acids but

to a lower degree. Fat exerted a mild influence. Soluble starch, maltose and dextrose elicited negligible effects. These observations coupled with the additional finding that atropinization did not prevent the pancreozymin effects of the peptones, caused the authors to conclude that the hormones, secretin and pancreozymin, play a dominant role in the regulation of pancreatic activity during normal digestion.

Magee and Anderson (94) found that fasted rats which were fed a high casein diet had more trypsin and lipase activity per gram of pancreas tissue dry matter, and more total pancreas dry matter than similar rats fed a diet high in gelatin, zein or glucose. They also found that daily urecholine injection and dl-valine supplementation to the diet increased the lipase and trypsin activities of the resting pancreas. They proposed that valine is a stimulant for the release of pancreozymin and that in the rat an adequate stimulus for the release of pancreozymin seems more important for lipase and trypsin production than the nutritional sufficiency of the protein consumed.

Magee and Hong (95) using similar methods as before (94) fed groups of rats a 7 percent casein diet supplemented with one of the following dl-amino acids; isoleucine, methionine, phenylalanine, leucine, lysine, or tryptophan. After a 3 week feeding period, the rats were fasted for 26 hours and pancreatic enzymes measured. They found that none of the amino acids studied affected amylase, methionine increased lipase and protease, and phenylalanine and isoleucine independently increased protease activity.

The same authors, Hong and Magee (61), using the same experimental

plan as above (95) supplemented a diet with an essential amino acid mixture prepared to be equivalent to an 8 percent casein diet. Other diets were prepared by omitting single amino acids from the mixture, or by the addition of single amino acids to a casein diet at 1.0 percent of the diet. By comparison with the pancreas enzymes of the control group, it was found that the addition of threonine decreased lipase, protease and amylase, arginine increased protease and histidine increased lipase. The complete essential amino acid mixture decreased protease. Omission of methionine or phenylalanine from the mixture decreased protease and omission of either isoleucine or valine decreased both lipase and amylase.

Magee and White (97) reported that rats fed alternately a high and low, 18.0 and 6.0 percent respectively, protein diet had significantly higher protease activities in the pancreas than the control rats fed either the high or the low protein diets.

Magee and Hong (96) have studied the effect of diet on the pancreas using chronic pancreatic fistula dogs in which the main duct was cannulated and the accessory duct ligated. Total daily secretion was collected for a 3 day control period, followed by a 3 day test period during which the dogs received the standard control diet supplemented with the experimental nutrient, which was followed again by a 3 day control period. The secretion was collected in sterile balloons containing glycerol and aliquots of the secretion were taken. They found that the protease activity of the secretion as collected was only 5 percent of the total activity after activation and that the volume and enzyme activity of the secretion could not be correlated with the body weight of the dog.

They suggested that the latter effect is due to the fact that varying fractions of the total glandular secretion were drained by the cannula. Doubling the quantity of control test meal invariably increased the volume, protease, and amylase output of the gland. Soya flour increased volume and amylase significantly but did not affect protease; peptones increased the volume of secretion significantly, but not the protease and amylase output. Neither defatted milk solids nor purified casein had a significant effect on enzyme or volume output. Corn oil caused large increases in the volume, amylase, lipase, and protease output of the pancreas. Addition of a casein hydrolysate was without effect on the secretion. Addition of essential amino acids produced evidence that the output of the individual enzymes need not be parallel and that the volume can be affected without the enzymes. Isoleucine, threonine and lysine increased amylase but not protease, methionine increased protease but not amylase, lysine increased amylase but not volume output. Phenylalanine increased the output of amylase, lipase, and protease and also the volume. Leucine, arginine, histidine, and tryptophan had no significant influence on the enzyme output.

Chernick et al. (16) observed that chicks consuming diets containing raw soybean meal, had hypertrophied pancreas glands and more proteolytic activity in the gland than control birds consuming an autoclaved soybean meal. Although supplemental methionine significantly increased the growth rate of the raw soybean meal fed chicks, it did not alleviate the increased size and proteolytic activity of the pancreas. They proposed that the trypsin inhibitor of the raw soybean meal was responsible for these

effects.

More recently Lyman and Lepkovsky (89) found that the lipase and amylase activity in the small intestine of rats increased immediately after ingestion of a 50 percent raw soybean meal diet. Three hours later the enzyme activities reached values 3 to 4 times that of similar control animals on a heated soybean meal diet. The activity of the enzymes in the pancreas tissue decreased coincident with the increased activity of the intestinal contents suggesting an increased enzyme secretion rate by the pancreas. Ingestion of crude and crystalline trypsin inhibitor preparations had very similar effects on the enzyme content of the pancreas and intestinal contents. The authors concluded that their data contest rather than support the concept that intestinal proteolysis is reduced by the presence of the trypsin inhibitor.

Lyman (88) found that rats fed a diet containing raw soybean meal or a diet containing heated soybean meal and a crude or crystalline trypsin inhibitor had greater activities of protease, amylase, and lipase in the intestinal tract than the controls and appreciable activity was found as far posterior as the colon. He concluded that the adverse effects of raw soybean meal could be due to the loss of essential amino acids by bacterial degradation of endogenous proteins, rather than due to a reduction of proteolysis.

Lepkovsky et al. (81) found that the proteolytic activity of the feces of chicks between 1 and 5 weeks of age could be almost entirely eliminated by ligation of the pancreatic ducts, demonstrating the pancreatic origin of the activity. They divided chicks into four groups of

eight birds each and measured the total proteolytic activity of the feces (24 hour collection) during a preliminary period and found that the four groups yielded similar values. All chicks were maintained on a 55 percent heated soybean meal diet during the preliminary period. One group was maintained on this same diet for 4 weeks, during this period the total activity of the feces gradually decreased. Methionine was added to the same diet of a second group of chicks which prevented the gradual decline of fecal activity observed in the first group. A third group was switched to a 55 percent raw soybean meal diet which caused a very marked drop in the total fecal activity to values of approximately 20 percent of the preliminary level. This marked drop was followed by a very gradual increase in the fecal activity during the 4 week period. The fourth group of chicks received the same diet as the third plus supplemental methionine; this group also demonstrated a marked initial decrease in the total proteolytic activity, but the drop was followed by a rapid increase so that after 4 weeks on this diet the total fecal protease activity was higher than the other 3 groups.

Lyman (90) studied the effect of specific amino acid deficiencies on pancreatic enzymes of rats. Groups of rats were made deficient in phenylalanine, threonine, histidine, or methionine. After 10 days on the deficient diets, rats of the respective groups were fed the diet alone or the diet plus a pancreatic stimulant. The lipase, amylase, and protease activities of the pancreas and intestine were determined 2 and 23 hours after the diets were offered to the animals. All deficiencies depressed pancreatic enzyme levels during normal stimulation, when compared to the

controls, and greatly reduced intestinal enzyme activities during periods of stimulated secretion. Pancreatic protease was least affected by threonine; amylase and lipase were most affected by a histidine deficiency. Phenylalanine, histidine and methionine deficiencies were most effective in depressing intestinal protease levels. Repletion of all enzymes 23 hours after forced stimulation was inhibited by all of the amino acid deficiencies. He concluded that single essential amino acid deficiencies reduce pancreatic enzyme reserves, with certain enzymes being affected more by one deficiency than another.

Vonk et al. (135) found that pigs fed 20 grams of chlortetracycline per ton of feed had significantly heavier pancreas glands, and significantly higher total protease and amylase activity in the pancreas gland than the control pigs with no chlortetracycline in their diet. The values were all adjusted for body weight by covariance analysis, since the antibiotic treated group of animals grew at a faster rate during the feeding period, and it was found that only the amylase difference yielded statistical significance. In a separate report Vonk et al. (134) reported that chlortetracycline fed pigs had significantly more amylase and cellulase but not protease activity per gram of dry matter of intestinal contents.

## EXPERIMENTAL

### General Objectives

The investigations reported herein were conducted with two primary objectives, namely, to determine the quantitative output of the digestive enzymes of the pancreas gland of baby pigs between 2 and 8 weeks of age and to determine the relative enzyme secretion rates of the pancreas of the baby pigs consuming either a diet in which the protein was provided almost entirely by dried skim milk or an equivalent diet in which the protein was provided almost entirely by solvent extracted soybean meal. A secondary objective underlying the two primary objectives was to determine if a dietary influence on the pancreas could or could not explain the well established differential in baby pig growth and efficiency of feed utilization for dried skim milk and soybean meal basal diets.

### General Approach

The experimental methods previously used for the study of exocrine pancreatic function have been summarized in the Review of Literature. Among the various methods reviewed, only the pancreatic fistula satisfies the first primary objective of this study; to determine the output of the pancreatic enzymes quantitatively. Therefore it was decided to use pancreatic fistulae in this study. It would have been desirable to use an experimental design in which an individual baby pig would be prepared with a pancreatic fistula at 1 week of age and after recovery from the

operative procedure, the secretion would be collected quantitatively at intervals up to 8 weeks of age, and all the secretion issued between collection periods returned to the duodenum to accomplish its normal digestive and buffering functions. The two diets could be alternated at sufficiently frequent intervals during the experimental period to reflect any possible dietary influences on the secretion. The number of animals needed for such an experimental design would be dictated by the experimental variations encountered.

It was realized, before the experimental investigations were undertaken, that the likeliness that this design could be attained was highly improbable. The following limitations and problems were acknowledged: the pancreatic fistulae used and described in the literature have inherent limitations which have already been reviewed (see Review of Literature); a comprehensive search of the literature did not reveal previous investigations in which the pancreas of the pig was drained by external fistulae; the baby pig has a very restless disposition in comparison to the dog, the most popular animal in this type of study due to its size, endurance, conduct and intelligence; the anatomical size of the baby pig and of its organs is much smaller than the adult dog and therefore probably would require special equipment not commonly accessible; the physiological age and development of the 2- to 8-week-old baby pig in comparison to the mature dogs used most commonly may present unique problems. Successful collection of the total pancreatic secretion at predetermined intervals without disturbing normal pancreatic digestion between sampling periods was approached most closely by Thomas (130) by

temporarily cannulating the pancreatic duct through a duodenal fistula, a procedure which requires that the papilla of Vater, an anatomic structure in the intestine surrounding the pancreatic duct of mature dogs but apparently absent or undifferentiated from intestinal tissue of the newborn pig, be clearly visible. In addition, the evidence presented in the Review of Literature clearly indicates that the exact mechanism by which a diet, its individual components, or its digestive products influence the pancreas is not at present fully understood. Therefore, frequent alternations of the two experimental diets might not allow the diets sufficient time to express their influences in the event the influence is exerted over a period of time as suggested by Pavlov (112), Magee and Hong (96), and Grossman et al. (43).

Regarding the analysis of the enzyme content of pancreatic tissue, a common procedure used in baby pig studies, it is the writer's opinion that the enzyme content of the pancreatic tissue is a function of two parameters; enzyme synthesis and enzyme secretion. Evaluating the rate of enzyme secretion into the duodenum, the most quantitative measure of the digestive function, would require that the history of the rate of enzyme synthesis and of enzyme storage or removal from the acinar cells immediately before the tissue was sampled and analyzed be known. It would appear that an evaluation of the latter two phenomenon would require techniques more cumbersome and less physiological than the external pancreatic fistula. Finally it is apparent from the evidence in literature that the enzyme content of pancreatic tissue and the rate of secretion of the individual enzymes via a fistula are highly variable.

By comparison the analysis of pancreatic tissue is an expression of the balance between the synthetic and secretory processes of the gland at the moment the tissue is sampled, whereas a successful fistula reflects the total work of the gland over the period of collection.

It was decided that due to the disposition of the baby pig it would have to be restrained, and special equipment was designed for this purpose. Animals near 8 weeks of age were fistulated first to give the investigator experience with a larger animal and to define the variations and limitations before attempting to use the small and immature 1-week-old pig.

As previously mentioned an alternative method, pancreatic duct ligation, was selected for supplementary investigation. It was recognized that the two primary objectives defined above could not be directly explored by this technique, but the relative role of the pancreas in the overall digestive process could be indirectly evaluated and dietary influences upon it investigated by establishing the degree of reduction of dietary protein and dry matter digestibility caused by the exclusion of pancreatic digestion with each of the two diets. This technique also has inherent limitations which were reviewed in the Review of Literature. In addition a few observations were made on the secretion of the anesthetized pig after stimulation with pancreozymin and secretin to determine if the glands of young pigs could be stimulated by the physiological hormones. The methods used and the development of the program are discussed in detail in the sections that follow.

### Animals

The animals used in these investigations were from the swine herd at the Iowa State University, Swine Nutrition Research Farm. For the most part, the animals were of crossbred breeding. The animals were farrowed in individual farrowing stalls. Generally the animals were weaned from their dams at 7 days of age onto their respective experimental diets. The animals were maintained for several days after weaning under infra-red heat lamps until it was observed they were eating well. Invariably assistance was required to help the animals start eating by forcefully depositing a small portion of the diet on the tongue. Within 24 hours after birth, each pig was individually weighed and ear notched, eye teeth clipped, and 100 milligrams iron, as iron-dextran, injected intraperitoneally. The male pigs were castrated at approximately 3 days of age.

### Diets

Two diets were prepared on the basis of the calculated composition of the foodstuffs and ingredients used to meet all the requirements of the baby pig recommended by the National Research Council (106) plus an additional allowance recommended by the Iowa State University, Swine Nutrition Staff. In the event that the calculated content of one ingredient exceeded the recommended allowance in one of the two diets, the content of the second diet was adjusted to the same level. The two diets used in this study were selected because of the well established difference in animal performance reported by numerous investigators using

dried skim milk and soybean meal basal diets with young pigs. For brevity these two diets will be referred to subsequently as the milk protein diet and soybean protein diet. The ingredient composition of the diets and the vitamin premixes are shown in Tables 1 and 2 respectively. The calculated analyses of the complete diets are shown in Table 3. From the values presented in Table 3 it can be seen that the two diets have almost identical nutrient composition, based on the reported values of Hubbel (62), Meyer et al. (103) and the analysis of the premix preparations. In addition to the balance shown in Table 3, lactose was added to the soybean protein diet to equal that added by dried skim milk to the milk protein diet, and corn starch was added to the milk protein diet in amounts approximating the plant starches contained in the soybean meal of the soybean protein diet. The total fiber of the two diets was balanced by the addition of wood flock to the milk protein diet. The ground yellow corn was added to the diets only to give the complete diet a coarse texture because it was found that without corn, the diets were of such a fine texture that they did not flow freely in conventional baby pig feeders. The corn provided 8.6 percent of the total protein in both diets, the remaining 91.4 percent was provided by dried skim milk and soybean meal in the milk protein and soybean protein diets respectively. It is presumed that the major difference between the two diets is the source of the protein.

Table 1. Composition of experimental diets.

Foodstuff or ingredient	Milk protein diet	Soybean protein diet
	%	%
Gr. yellow corn	20.00	20.00
Corn starch	15.83	3.05
Sucrose	4.90	5.66
Lactose		26.90
Dried skim milk	53.50	
Soybean meal (50% protein)		36.70
Stabilized lard	2.00	2.10
Woodflock	1.16	
Vitamin premix	2.00	2.00
Iodized salt	0.10	0.74
Calcium carbonate		0.21
Dicalcium phosphate		1.94
Trace minerals <sup>a</sup>	0.20	0.20
KCl		0.50
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.31	
	<hr/>	<hr/>
Total	100.00	100.00

<sup>a</sup>Trace elements contained at the following percentages: Fe, 7.0; Cu, 0.475; Co, 0.166; Zn, 8.10; Mn, 5.68; Ca, 5.28; K, 0.750.

Table 2. Composition of vitamin premix.

Vitamin	Potency	Total amount per 2 lb. <sup>a</sup>	
		Milk protein diet	Soybean protein diet
Vitamin A	20,000 I.U./g.	13.01 g.	13.50 g.
Vitamin D	142,000 I.U./g.	0.28 g.	0.35 g.
Riboflavin	Crystalline	8.00 mg.	446.00 mg.
Pantothenic acid	Crystalline	153.00 mg.	736.00 mg.
Niacin	Crystalline	2.53 g.	2.45 g.
Choline	70 percent	29.94 g.	
Vitamin B <sub>12</sub>	0.10 percent	0.662 g.	2.00 g.
Alpha tocopherol	20,000 I.U./lb.	21.80 g.	21.72 g.
Ascorbic acid	Crystalline		1.70 g.
Vitamin K (Menadione)	Crystalline	50.00 mg.	50.00 mg.
Thiamine	Crystalline	184.00 mg.	230.00 mg.
Pyridoxine	Crystalline	103.00 mg.	20.00 mg.
Folic acid	Crystalline	32.00 mg.	37.00 mg.
Biotin	0.01 percent		2.51 mg.
Butylated hydroxytoluene	25 percent	11.35 g.	11.35 g.
Corn starch		<u>827.90 g.</u>	<u>853.35 g.</u>
	Total	908.00 g.	908.00 g.

<sup>a</sup>Two lbs. of Premix were contained in each 100 lbs. of the complete diets.

Table 3. Calculated analysis of the complete experimental diets.

Constituent	Milk protein diet	Soybean protein diet
Protein %	20.01	20.09
Fat %	3.05	3.04
Fiber %	1.69	1.61
Vitamins:		
Vitamin A, I.U.	3000.0	3000.0
Vitamin D, I.U.	500.0	500.0
Riboflavin, mg.	5.0	5.0
Pantothenic acid, mg.	10.0	10.0
Niacin, mg.	30.0	30.0
Choline, mg.	517.1	517.1
Vitamin B <sub>12</sub> , mcg.	20.0	20.0
Alpha tocopherol, mg.	10.0	10.0
Ascorbic acid, mg.	17.01	17.01
Vitamin K (Menadione), mg.	0.5	0.5
Thiamine, mg.	3.0	3.0
Pyridoxine, Mg.	2.0	2.0
Folic acid, mcg.	500.0	500.0
Biotin, mcg.	86.2	86.2
Minerals:		
Cl, %	0.781	0.714
Na, %	0.310	0.298
K, %	1.0	1.012
Mg, %	0.117	0.12
Ca, %	0.693	0.703
P, %	0.61	0.63
Fe, %	0.0171	0.0194
Cu, mg.	7.47	7.08
Co, mg.	1.51	1.51
Zn, mg.	73.55	73.55
Mn, mg.	52.61	56.66
S, %	0.195	0.182

## Equipment and Materials

### Partial restraining collection unit

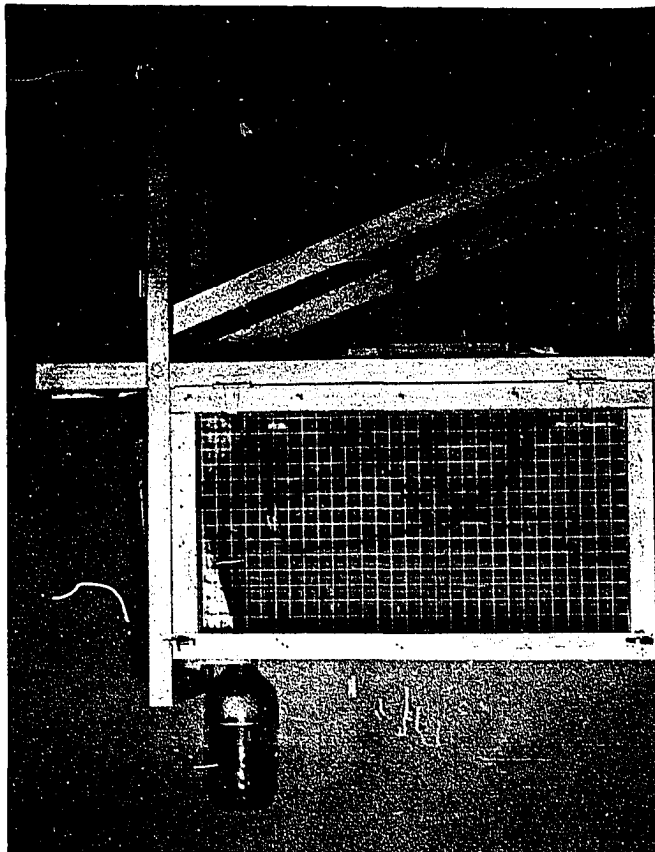
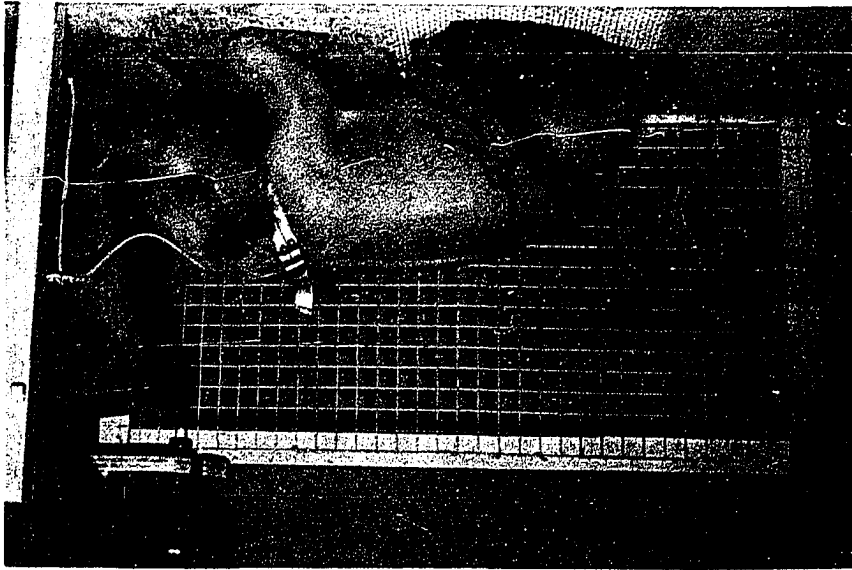
A unit was designed for the maintenance of chronic pancreatic fistula pigs which after several modifications performed satisfactorily. For brevity the partial restraining collection unit will be subsequently referred to as the collection unit. See Figure 1 for a photograph of the unit. The animal was restrained within the unit by the wire cloth partitions laterally, the feeder and waterer anteriorly and the end gate posteriorly. The animal was further restrained by use of a harness which limited animal movements and kept the animal oriented in a posterior-anterior direction.

The harness prevented the animal from making complete turns and from making excessive movements during collection of the pancreatic secretion. It further limited the movements of animals so that male animals (male animals were required) urinated over the area of the urine collection funnel. Yet it allowed sufficient liberty for the animal to lie down. The harness served still another function, to support the plastic feces collection bag over the anus to facilitate direct collection of feces. Continuous collection of feces was accomplished simply by replacing the contaminated plastic bag with a clean bag. See Figure 2 for a photo of pig in the harness.

Urine was collected in a glass vessel suspended under a 10 inch diameter glass funnel mounted under the wire mesh opening in the floor. This arrangement can be seen in Figure 2.

Figure 1. Photograph of partial restraining collection unit.

Figure 2. Photograph of a baby pig restrained by a harness in the collection unit.



### Secretin

The hormone, secretin, was used in this series of experiments. It was obtained from The Lilly Research Laboratories, Indianapolis, Indiana. It was prepared from hog duodena and standardized by a method similar to that used by Ivy (66) by the manufacturers. The preparation had a potency of 1.67 clinical or Ivy dog units per milligram.

### Pancreozymin

The hormone, pancreozymin, was also used in these investigations. It was purchased from Boots Pure Drug Limited, Nottingham, England, in vials containing 100 units. The preparation contained cholecystokinin.

## Methods

### Chronic pancreatic fistula

In the series of experiments following where the pancreatic duct was cannulated, the cannulation procedure used was as follows: the animal was anesthetized with sodium pentobarbital by a small initial intraperitoneal injection of the anesthetic because it permitted rapid injection with a minimum of excitation and struggle of the pig. After the animal became physically incapacitated from the initial injection, the anesthesia was carried to the surgical level by slow intravenous (external jugular) injection. The animal was further prepared for surgery by clipping all hair at the site of the laparotomy, followed by shaving the same area and the whole body scrubbed with soap and warm water. The surgical site was then sponged with ethyl alcohol and surgery

followed immediately after the site was isolated with sterile towels. The pancreatic duct was exposed by an incision approximately 9 centimeters in length on a line parallel with the posterior-ventral margin of the rib cage on the right side. The laparotomy penetrated the integument, the cutaneus trunci, obliquus abdominus externus, obliquus abdominus internus, and transversus abdominus muscles, the fascia transversalis, and the parietale peritoneum, thus exposing the viscera.

The pancreatic duct was exposed by placing two ligatures around the duodenum, one on each side of the pancreatic duct, and gently securing the duodenum in such a way that the duct is easily accessible. A polyethylene cannula manufactured by Clay Adams, Inc.<sup>a</sup> (tested by same and found to cause no tissue reaction) was used. The cannula was prepared from a six inch (approximately) piece of the material of proper diameter. A slight expansion in the cannula was created by gentle heat applied to the area selected for the expansion. The function of the expanded area was to facilitate securing the cannula into the duct by ligatures.

Once the cannula was placed into the duct and secured by ligatures, the distal end was exteriorized via a stab wound at a site on the line of the costo-chondral junction and near the dorsal limits of this region. Keeping the cannula in a dorsal position prevented the animal from lying on the structure and also minimized harness irritation. Surgery control animals were prepared in some of the experiments by performing the entire

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<sup>a</sup>Clay Adams Inc., 141 East 25th Street, New York 10, N. Y.

operation outlined above with the exception of the cannulation detail. The laparotomy was closed with 4 rows of continuous sutures and the animal was transferred to a warm location. An infra-red heat lamp provided warmth for the animal for about 24 hours post-surgery. The method has many similarities to the method of Elman and McCaughan (33) and the method used by Magee and Hong (96).

A duodenal fistula was established in a few animals using the same polyethylene cannula material of such a size that the cannula of the pancreas fit tightly into the luminal diameter of the duodenal cannula. It was hoped that this would permit the pancreatic secretion to return to the duodenum during those periods that the secretion was not being collected, and therefore alleviate the chronic involvements observed after continued drainage of the entire secretion. It was found however that the pressure within the duodenum oscillates with peristaltic activity and frequently this pressure was greater than the pressures in the pancreas with the result that the expected direction of flow was frequently reversed, which produced acute and intolerable consequences.

In a further attempt to return the pancreatic juice issued via the fistula to the duodenum, an isolated segment of the duodenum which received the pancreatic duct and therefore the pancreatic secretion was fistulated by a polyethylene cannula (see Dragstedt et al. [28]) which was joined to a second duodenal fistula after the continuity of the digestive tract was reestablished by end to end anastomosis. This was found to be a feasible and workable arrangement. However the operative procedures had a critical influence on the animal. Vomiting was common

on a liquid milk diet and the dry experimental diets were accepted in such small quantities that it was decided not to use the method further.

The details of the surgical techniques followed in the preparation of these fistulae were generally those outlined by Markowitz et al. (100).

#### Pancreatic duct ligation

The surgical procedure followed for the ligation of the pancreatic duct was the same as that described above for the preparation of the pancreatic fistula, except that after the pancreatic duct was exposed, two nylon ligatures were tightly placed around the duct, and the laparotomy closed. Sham operated control animals were prepared in the same way without placing the ligatures on the duct. The post-surgical care of the animals was the same as for animals prepared with a fistula.

#### Collection of pancreatic juice

The pancreatic juice was collected at intervals by attaching one end of a cannula extension to the exterior portion of the pancreatic duct cannula and the other end was fitted to a clean graduated test tube which was securely positioned in an erlenmeyer flask containing ice water, through a rubber stopper so that only the upper two inches of the tube was not submerged in the ice water. The collection tube and ice bath assembly were then positioned so that the exit tip of the extension cannula was six inches above the floor of the collection unit. This way, after the extension cannula filled with secretion the pancreas was secreting against a slight negative pressure, the negative pressure varying with the height of the animal and also with postural changes of

the animal.

To justify this procedure, Grossman (42) found that partial obstruction of the pancreatic duct did not depress the volume of secretion or the protein output of the gland until the obstruction pressure was near the maximum secretory pressure. He did not investigate the effects of negative pressures.

The animals were observed constantly during the collection of secretion and the periods of collection were timed with a laboratory interval timer.

The samples were stored in an ice chest until they could be transferred to a freezer chest, where they were stored if the analyses were not done immediately. In most cases however, the analyses were started the following day, and the samples were stored in a refrigerator. The freezer chest maintained the temperature at  $-10^{\circ}\text{C} \pm 2^{\circ}$ , the refrigerator at about  $4^{\circ}\text{C}$ .

#### Analysis of pancreatic juice

The pancreatic juice in these experiments was analyzed for one or all of the following: buffering capacity, amylase, lipase and protease activities. The methods are described below.

Buffering capacity This was determined by titrating exactly 1.0 milliliter of pancreatic juice diluted in 10 milliliters of distilled water to the phenolphthalein end point with 0.005 N hydrochloric acid. The milliequivalents of acid required for this titration was calculated. Thus the buffering capacity of the secretion is expressed as

milliequivalents HCl per milliliter of pancreatic juice.

Amylase activity      The method used for the analysis of amylase activity of pancreatic juice is included in this dissertation in its entirety, see Appendix I. The method is a modification of the method of MacLeod and Robison (91) and is very similar to the method of Willstatter which is described by Sumner and Somers (126, p. 83). It was found by plotting curves that the milligrams of maltose liberated using this method was directly proportional to the quantity of a high potency animal diastase preparation until the total maltose liberated in the digest was calculated to be more than 295 milligrams, 14.75 percent of the starch substrate, after which the proportionality and sensitivity of the curve were adversely affected.

The pancreatic juice was diluted with the same phosphate buffer as was used in the analytical analysis until the final result was within the range of linearity. Generally the unknown values fell within the range of linearity using 1.0 milliliter of a 1 to 50 dilution of pancreatic juice. If the total amount of maltose liberated was in excess of 290 milligrams the pancreatic juice was diluted more and the analysis repeated.

The amylase activity is expressed as milligrams maltose equivalents per milliliter of undiluted secretion. All values are averages of duplicate analyses.

Lipase activity      The lipase activity of pancreatic juice was determined by the method of Balls, Matlack and Tucker, outlined by Sumner and Somers (126, p. 55) using a tributyrin substrate and a digestion period of one hour. Preliminary use of the method revealed a

linear relationship over a wide range between the milliequivalents of potassium hydroxide required to titrate the fatty acids liberated and the quantity of a pancreatic lipase preparation used. All the unknown values obtained using 1.0 milliliter of pancreatic juice diluted 1 to 10 fell within the range of the linear preliminary curve. Lipase activity is expressed as milliequivalents potassium hydroxide per milliliter of undiluted pancreatic juice. All values reported are the averages of duplicate determinations.

Protease activity      The protease activity of pancreatic juice was determined by the colorimetric ninhydrin procedure outlined by Davis and Smith (26). Since this procedure outlines only the general principles of the method and suggested analytical maneuvers it is pertinent to include the specific procedure used here. A 1.00 percent casein substrate was prepared as described by Vonk et al. (133) in a sodium hydroxide solution. A portion of the stock solution was titrated to pH 7.0 and diluted to a concentration of 0.5 percent casein with distilled water before it was further diluted to a final concentration of 0.04 percent casein with the phosphate buffer.

The pancreatic juice was diluted 1 to 25 or more with the 0.01 molar phosphate buffer at pH 7.0 and the trypsinogen and chymotrypsinogen were activated by incubating 1.0 milliliter of the diluted pancreatic juice with 1.0 milliliter of an active trypsin solution, containing 0.01 milligrams of activated trypsin also in the 0.01 M phosphate buffer, for ten minutes in a water bath set to hold the temperature at 40° C.

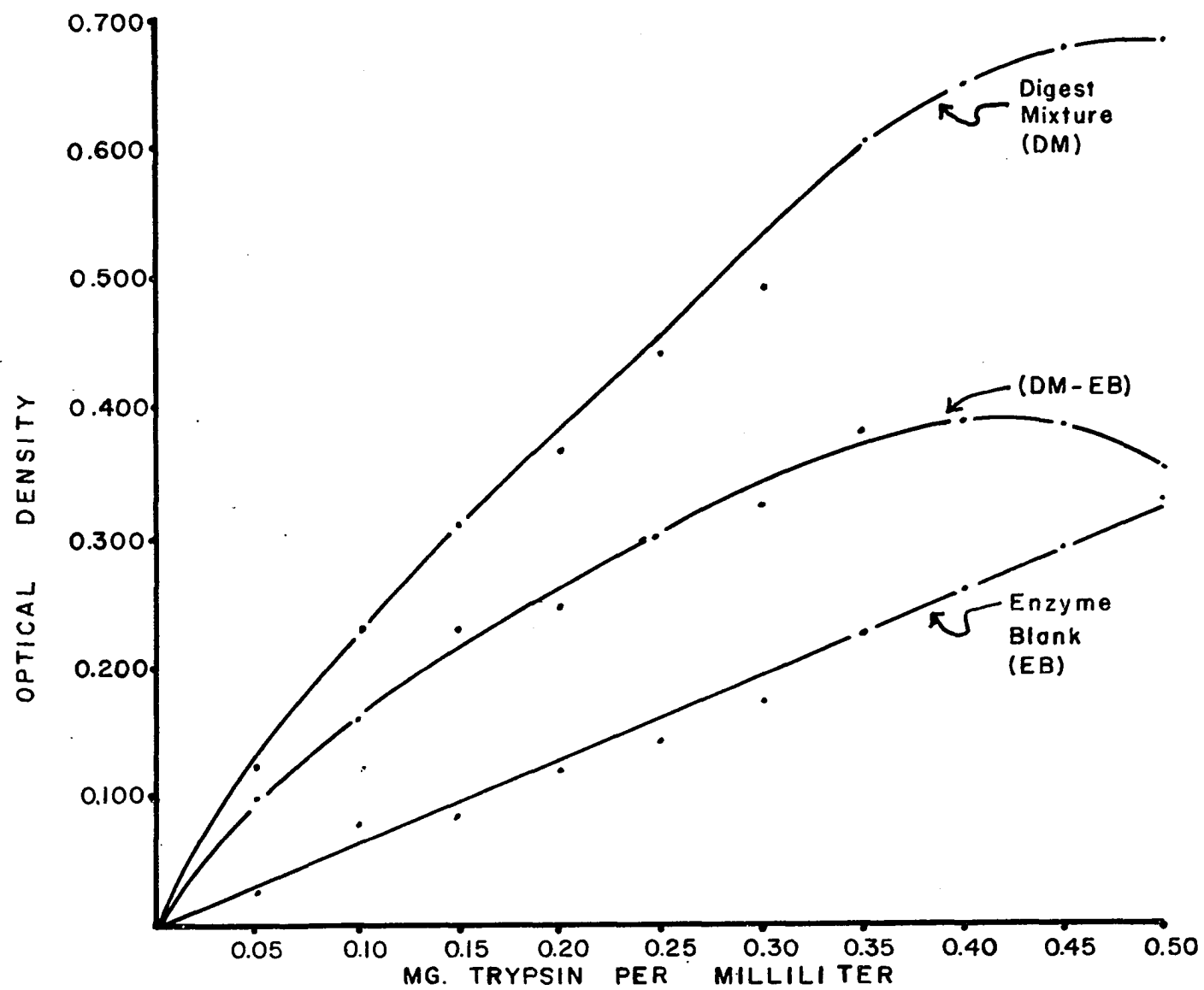
Immediately after the enzyme activation was completed 1.0 milliliter of the pancreatic juice-activator-mixture was transferred to an identified test tube containing 9.0 milliliters of 0.04 percent buffered casein substrate solution already in the water bath at 40° C., mixed thoroughly and the time recorded. An activator blank was prepared in the same way using 0.5 milliliter of the activator solution and 0.5 milliliter of the 0.01 M buffer in place of the pancreatic juice-activator-mixture. The total volume of the digest mixture in all cases was 10.0 milliliters. Immediately after this 0.1 milliliter of the same pancreatic juice-activator-mixture, the quantity contained in 1.0 milliliter of the digest mixture, was transferred to another tube containing 1.0 milliliter of ninhydrin solution and 0.9 milliliters of the 0.01 M phosphate buffer to be used as the enzyme blank. Thirty minutes after the activated pancreatic juice was added to the digest mixture, a 1.0 milliliter aliquot was quickly transferred to a second tube containing 1.0 milliliter of ninhydrin solution. The ninhydrin was prepared as described by Moore and Stein (104). The ninhydrin tubes were thoroughly mixed and the color developed by heating the tubes in a vigorously boiling water bath for 20 minutes and the optical density was determined after dilution of the boiled ninhydrin mixture with 5 milliliters of a 50:50 mixture of ethanol and water using a Beckman Model DU spectrophotometer at a wavelength of 570 millimicrons. The spectrophotometer was set at zero optical density against the color intensity of 0.05 milliliter of the activator solution and 0.95 milliliter buffer for the reading of the enzyme blanks, and against the color intensity of the activator blank for reading the

optical density of the digest mixture.

Instead of preparing an amino acid standard curve and expressing the results in terms of amino acid equivalents as suggested by Davis and Smith, a standard curve was prepared by replacing the volume of the pancreatic juice as used in the method above with known amounts of active trypsin. The standard curve (see example in Figure 3) used for the interpretation of the pancreatic juice activities was constructed by plotting the difference of the optical densities (DM-EB) of the digest mixture (DM) and the enzyme blank (EB) against the concentration of active trypsin in standard solutions, and the activity of the pancreatic juice was determined directly from the curve. The activity is expressed as milligrams trypsin equivalents per milliliter of undiluted pancreatic juice.

The addition of 0.90 milliliter of the 0.04 percent casein substrate to each of the enzyme blank tubes with the ninhydrin instead of the buffer, and all the tubes read with the spectrophotometer set at zero optical density against an activator blank (prepared as described above, except that the casein substrate and the activator were added directly and separately to the ninhydrin so that no digestion would occur) demonstrated that a very slight amount of casein was digested by the activator and that after correction for the slight increase in optical density by subtraction, the analytical interpretation curves were identical. Further, if the enzyme blanks were evaluated by removing a 1.0 milliliter aliquot immediately after the standard enzyme solutions were added to the casein substrate at 40° C., as suggested by Davis and

**Figure 3. Example of protease standard curve.**



Smith, it was found that the optical density plot was not linear, as for the enzyme blank (EB) line represented in Figure 3, and the slope of the plotted line increased with increasing trypsin activity suggesting that some digestion had already occurred. This justifies the procedure used here of evaluating the optical density of the enzyme blanks before the enzyme preparation was added to the casein substrate.

Pancreatin (3 X USP) was used as the standard reference in Experiment 1026, and activated trypsin in all other experiments, otherwise the method was the same.

#### Pancreozymin-secretin function test

This test was used to a limited extent near the termination of this program. The animals were fasted for a period of 24 hours and then the pancreatic duct and duodenum were isolated by the same surgical procedures described for the preparation of pancreatic fistulae. A Doyen intestinal clamp was placed on the duodenum approximately 3 centimeters anterior to the point the pancreatic duct enters the intestinal wall. The intestinal juice and contents were forced posteriorly from the Doyen clamp, clearing the lumen of the intestine for a length of approximately 20 centimeters. The mucosa of the duodenum was exposed by a 1 centimeter long incision about 15 centimeters posterior to the Doyen clamp. A polyethylene cannula with an internal diameter of 0.062 inch, fitted with an enlarged polyethylene end piece with numerous perforations, was inserted through the incision into the lumen of the duodenum so that the perforated end piece was in the immediate area where the pancreatic juice was secreted. A ligature was then placed around the duodenum with the cannula in such a

way that an isolated segment of the duodenum was drained by the cannula. The edges of the wound were then brought together and clamped with the free end of the cannula extending from the wound. The free end was then fitted to a graduated test tube using a rubber stopper already fitted with a vacuum line. The vacuum line was connected to a large air tight vessel with a mounted mercury manometer for adjustment of the negative pressure inside. The graduated tube was then placed into a flask of ice water. At this point the pancreozymin suspended in sterile physiological saline was injected into the external jugular vein and followed immediately by secretin also in sterile physiological saline. The vacuum in the collection tube was then adjusted to -1.0 centimeters of mercury and the secretion collected for the desired time. The vacuum was to prevent the very viscous fluid, primarily intestinal mucus which fills the cannula first, from blocking the cannula. The pancreatic juice was then analyzed by the procedures outlined above.

The animal was maintained under surgical general anesthesia the entire duration of the test. Immediately upon completion of the test, the clamps, ligature, and cannula were removed after the sterile sponges, which were used to "pack off" the duodenum at the site of the incision to prevent possible contamination by duodenal drainage, had been removed. The duodenal incision and laparotomy were then sutured. The post-operative care of the animal was described above.

This method is similar to the method described by Sun and Shay (128) in which the duodenal contents of human patients were aspirated after administration of pancreozymin and secretin.

Digestibility of protein and dry matter, biological value, and nitrogen retention

In Experiments 1074, 1085, 1099, and 1114, protein and dry matter digestibility, dietary protein biological value, and nitrogen retention were evaluated. In accordance the feces and urine were collected quantitatively over a predetermined period and the quantity of feed represented by these excreta were assessed as accurately as possible. In Experiments 1074 and 1085 the animals were fed a limited and constant amount of feed twice daily, in these two experiments the collection of feces and urine were started immediately before one of the meals was given and terminated at the same time with respect to another meal at the end of the desired collection period. The digestibility, biological value, and nitrogen retention values were calculated on the basis of the total feed consumed between the time the feces and urine collection were initiated and terminated. This procedure is expedient because the feed consumption was relatively constant and was consumed with more regularity than animals allowed feed ad libitum. Also in these same two experiments, male animals were used in the collection unit designed for this study and there was perfect separation of feces and urine, which unfortunately cannot be accomplished with conventional digestibility equipment for baby pigs.

In Experiment 1099, the animals were maintained in conventional baby pig digestibility stalls. The collection period was initiated by feeding the animals a very small portion of a "marked" diet which was prepared by thoroughly mixing 1.0 pound of chromium oxide into 99.0 pounds of the

respective experimental diets. A diet prepared in this way also retains the green color upon excretion of the indigestible residues in the feces, thus referred to as "marked" feces. After the animals consumed of the "marked" feed, the "unmarked" experimental diet was offered and at the end of the collection period the total amount of the "unmarked" diet consumed was recorded and again the "marked" diet was offered. The digestibility values were calculated on the basis of the total feed consumed and the total feces excreted between the two "markers". Urine was collected over a period which coincided exactly with the period between the two diet "markers". It was found that due to the extremely fine texture of the experimental diets and the design of the digestibility stalls that the urine was contaminated by feed and feces. Also the feces of some animals was contaminated with feed. Also judging from the amount of feed which collected on the urine pan, but was not washed down into the urine collection vessel, the total feed consumption values were in slight error. All wasted feed which was clearly distinguishable from the excreta was recovered and the consumption values corrected accordingly.

In Experiment 1114 the collection period was initiated by inserting the feces and urine collection pan into the digestibility stall 24 hours after completion of the surgical operation. The animals were fed their respective experimental diets which contained 0.50 percent chromium oxide thoroughly mixed into the diet throughout the collection period. All the feces and urine were collected for 7 days from the 2-week-old group of animals and for 5 days from the 4- and 6-week-old groups of animals. The

quantity of the diet represented by the feces was calculated on the basis of the total chromium collected in the feces and the concentration of the chromium in the experimental diets for calculation of the digestibility values. Thus the grams of dry matter per gram chromium in the diet multiplied times the total grams of chromium collected in the feces was taken as the dietary dry matter equivalent in the feces and the digestibility was calculated. The dietary nitrogen equivalent of the feces, required to calculate the protein digestibility, was calculated by multiplication of the dietary dry matter equivalent of the feces times the nitrogen per gram of dry matter in the diet.

The experiments in which digestibility, biological value, and nitrogen retention were determined; the diet was prepared fresh for each study, feces was collected twice daily and the total collection frozen in plastic bags, urine was collected daily and the total volume determined and an aliquot taken and transferred to the freezer except in Experiments 1099 and 1114 the urine was transferred to a large vessel and stored in closed containers under toluene at room temperature. The total volume was determined at the end of the collection period and a sample removed for analysis.

The nitrogen content of the feces, urine and diet were determined by the standard micro-Kjeldahl distillation procedure using boric acid in the distillate receiving vessel. All analyses were done in duplicate using approximately 100 milligrams of the dry matter of feces and diet and 0.5 milliliter of urine. The averaged values are expressed in this dissertation.

The dry matter content of the diet and feces was determined by drying the feed samples and the total quantity of feces collected to a constant weight at 80° C. in a constant temperature oven.

The dry matter of feed and feces was finely ground in a Wiley mill and mixed thoroughly and a sample was transferred to a desiccator until removed for nitrogen analysis.

The chromium content of the diet and feces was determined by the colorimetric method of Kimura and Miller (73) on duplicate samples of approximately 100 milligrams of feces dry matter and 300 milligrams of diet dry matter. The duplicate values were averaged and are reported herein.

#### Histopathological and gross observation of experimental animals

In Experiments 1085, 1099, and 1114, some or all of the animals were taken to the Iowa State University, Veterinary Diagnostic Laboratory to be observed for gross lesions and histopathologic alterations. The animals and tissues were prepared and observed by a trained specialist.

The animals were subjected to the routine procedures followed by the Veterinary Diagnostic Laboratory. In general the animals were electrocuted and the carcasses and viscera were observed for gross lesions. The pancreas was removed approximately 30 minutes after the animals were euthanized and stored in a solution of 10 percent formalin in physiological saline. Three specimens of pancreatic tissue were taken at random from the anterior, median, and posterior sections of the gland. These specimens of pancreatic tissue were then trimmed and processed in an

Autotechnicon<sup>a</sup> according to the method outlined by Strafass (125, pp. 16-18), who also described the procedure used for imbedding the tissue blocks in paraffin and the sectioning of the tissues for preparation of the slides. The tissue sections were stained with Mayer's hematoxylin and counter stained with an alcoholic solution of ethyl eosin also in an Autotechnicon.

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<sup>a</sup>The Technicon Company, Chauncey, New York, U. S. A.

## INVESTIGATIONS

Eight separate experiments are reported in this section and each experiment is identified here by a number, the number corresponding to the experiment number under which the original data are filed by the Iowa State University, Swine Nutrition Section, Animal Husbandry Department. The experiments are reported in the sequence in which they were completed; the sequence of completion was not necessarily consistent with the number sequence.

## Experiment 1089: Baby Pig Performance on the Experimental Diets

Purpose

The purpose of this experiment was to determine whether the growth rates and feed efficiencies of baby pigs fed the two experimental diets would reflect the milk protein and soybean protein differential and the reduction of this differential as the animals approached 6 weeks of age as reported by other investigators. This test was essential in consideration of the experimental objectives of the overall program and the carefulness with which the two diets were balanced with respect to each other.

Procedure

Forty baby pigs were weaned from their dams at 7 days of age and transferred to an isolated brick building with 10 floor pens. A randomized block design was employed consisting of five blocks with two experimental diet treatments. Pairs of littermate pigs were randomly

allotted across the five blocks with one member of the pair on the soybean protein diet and the other member of the same pair on the milk protein diet. Each individual pen contained four unrelated animals but the two pens representing the two diets within each block contained four pairs of littermate animals. In the final analysis the pen was considered the experimental unit and accordingly the total gain and feed consumption were averaged for the four pigs and this average value was used for the statistical analysis of the data by the methods of Snedecor (124). Two animals were removed from the experimental design before the test was initiated; therefore two pens were represented by three instead of four animals and no corrections were necessary. The experiment was initiated when the pigs were 10 days of age and terminated after 35 days. The animals were individually weighed each 7 day period and the feed consumed by each pen was evaluated at the same time. Feed and water were available to the animals ad libitum. After the 35 day animal weights were recorded, feed and water were removed for a period of 24 hours and the animals were reweighed to determine the degree of body weight loss for the animals on the two diets.

#### Results and discussion

The average pig gains for each 7 day period and the total 35 day gains are summarized in Table 4, feed consumption data are summarized in Table 5, and the feed required per pound of gain, calculated directly from Tables 4 and 5 are summarized in Table 6. It was found that the pigs fed the soybean protein diet lost significantly more weight during

Table 4. Summary of average seven day gains.<sup>a</sup> Experiment 1089

Replication	Experimental period (days)					Total 35 day gain
	1-7	8-14	14-21	22-28	29-35	
Soybean protein diet						
1	0.02	0.44	3.25	4.56	8.19	16.46
2	0.07	0.31	2.31	5.25	8.19	16.13
3	-0.01	0.88	2.87	4.88	7.62	16.24
4	0.01	1.09	3.08	4.00	6.17	14.35
5	-0.06	0.88	3.81	5.25	8.38	18.26
Average	0.006	0.72	3.06	4.79	7.71	16.29
Milk protein diet						
1	0.58	1.75	4.09	7.66	7.67	21.75
2	0.84	0.81	3.63	6.50	8.75	20.53
3	0.59	1.19	4.12	6.75	8.81	21.46
4	0.56	1.00	3.06	5.00	6.69	16.31
5	0.48	0.94	4.00	6.31	8.63	20.36
Average	0.61	1.14	3.78	6.44	8.11	20.08
Soybean protein diet/milk protein diet - ratio						
	0.01	0.63	0.81	0.74	0.95	0.81

<sup>a</sup>All data on a per animal basis.

Table 5. Summary of average seven day feed consumption.<sup>a</sup> Experiment 1089

Replication	Experimental period (days)					Total 35 day consumption
	1-7	8-14	15-21	22-28	29-35	
<hr/>						
Soybean protein diet						
1	1.69	2.31	4.81	7.63	11.31	27.75
2	1.94	1.75	4.38	7.00	12.06	27.13
3	1.65	2.31	5.00	9.94	11.88	30.78
4	1.44	1.25	4.67	7.08	10.83	25.27
5	2.06	2.19	5.63	8.81	12.69	31.38
Average	1.76	1.96	4.90	8.09	11.75	28.46
Milk protein diet						
1	1.63	2.67	5.58	10.50	12.75	33.13
2	1.56	2.31	4.50	9.44	13.88	31.69
3	1.75	1.69	5.31	10.25	14.19	33.19
4	1.63	1.88	4.13	7.75	11.44	26.83
5	1.63	1.75	5.19	9.50	15.63	33.70
Average	1.64	2.06	4.94	9.49	13.58	31.71

<sup>a</sup>All data on a per animal basis.

Table 6. Summary of feed required per pound of gain. Experiment 1089

Replication	Experimental period (days)					Total 35 day period
	1-7	7-14	15-21	22-28	29-35	
Soybean protein diet						
1	84.50	5.25	1.48	1.67	1.38	1.69
2	27.71	5.65	1.90	1.33	1.47	1.68
3	-165.00	2.63	1.74	2.04	1.56	1.90
4	144.00	1.15	1.52	1.77	1.76	1.76
5	-34.33	2.49	1.48	1.67	1.51	1.72
Average	292.67 <sup>a</sup>	3.43	1.62	1.70	1.54	1.75
Milk protein diet						
1	2.81	1.53	1.36	1.37	1.66	1.52
2	1.86	2.85	1.24	1.45	1.59	1.54
3	2.97	1.42	1.28	1.52	1.61	1.55
4	2.91	1.88	1.35	1.55	1.71	1.65
5	3.40	1.86	1.30	1.51	1.81	1.66
Average	2.79	1.91	1.31	1.48	1.68	1.58
Soybean protein/milk protein ratio						
	104.90	1.85	1.24	1.15	0.92	1.11

<sup>a</sup>Calculated from total gains and feed consumption from the five replications because two replications lost weight.

the terminal 24 hour starvation period than pigs fed the milk protein diet, which suggests that before the starvation the soybean protein fed pigs had appreciably more feed and/or water in their digestive tracts than the pigs fed the milk protein diet. This invariable difference in body weight loss during the 24 hour starvation period was expected because it was observed during the greater part of the experiment that the soybean protein fed pigs had somewhat distended abdomens in comparison with the pigs fed the milk protein diet. It was decided on the basis of this observation to include the 24 hour fast period in the experimental procedure. Due to the consistent difference between the two diets in the terminal body weight loss, it was decided that the total body weight gain and the feed required per pound of gain during the 35 day feeding period would be calculated on the basis of the shrunk weights. The values for the total 35 day gains and feed efficiencies calculated on the basis of the shrunk weights were analyzed statistically and it was found that pigs fed the milk protein diet grew significantly faster and required significantly less feed per pound of gain than pigs fed the soybean protein diet.

The average pig gain, feed consumption, and feed required per pound of gain for the entire 35 day period are summarized in Table 7. The gains calculated on the basis of the nonfasted terminal weights and the percent body weight loss during the 24 hour fast period are also included in Table 7.

It can be seen that the pigs fed the milk protein diet gained more weight and required less feed per pound of body weight gain during each

Table 7. Summary of weight gains, feed consumption, and feed efficiency for the 35 day period, and of body weight loss during 24 hour terminal fast. Experiment 1089

Diet	Replication					Av.	$\frac{\text{SPD}}{\text{MPD}}$ ratio <sup>a</sup>
	1	2	3	4	5		
Total gain per pig (no terminal fast)							
Soybean protein	16.46	16.13	16.24	14.35	18.25	16.29	0.85
Milk protein	21.75	20.52	16.21	16.30	20.36	19.03	
Total gain per pig (based on 24 hr. terminal fast)							
Soybean protein	14.09	12.94	13.74	12.10	15.14	13.60	0.76 <sup>b</sup>
Milk protein	19.58	18.28	19.21	14.68	17.99	17.95	
Percent body weight loss during 24 hr. terminal fast							
Soybean protein	10.35	14.01	11.08	11.30	12.53	11.85	1.48 <sup>b</sup>
Milk protein	7.54	8.29	8.09	7.34	8.82	8.02	
Total feed consumed per pig							
Soybean protein	27.75	27.13	30.78	25.27	31.38	28.46	0.90
Milk protein	33.13	31.69	33.19	26.83	33.70	31.71	
Feed per lb. of body weight gain (based on 24 hr. terminal fast)							
Soybean protein	1.97	2.10	2.24	2.09	2.07	2.09	1.17 <sup>b</sup>
Milk protein	1.72	1.73	1.73	1.83	1.87	1.78	

<sup>a</sup>Soybean protein diet/milk protein diet ratio.

<sup>b</sup>Difference between diets significant ( $P < 0.01$ )

of the 5 weekly intervals with one exception (Tables 4 and 6). The data shown in Tables 4, 5, and 6 for the 29th through the 35th experimental days were calculated using the nonfasted 35 day final animal weights and therefore the values can be compared directly to those values calculated for the previous 4 weekly periods. Although the pigs fed the milk protein diet surpassed the pigs on the soybean protein diet in both expressions of performance, it can be seen from the soybean protein/milk protein ratios calculated in Tables 4 and 6 at each weekly interval, that the relative difference is greatest the first week and decreases each subsequent week so that at the end of the fifth week the animals on the two diets are performing very similarly. This is illustrated in Figure 4. The feed efficiency was plotted as the reciprocal of the soybean protein/-milk protein diet ratio for feed required per pound of gain so that the relative changes in feed efficiency and body weight gain could be more clearly visualized. With the exception of the fourth week of the experimental period it is clearly demonstrated that the performance of animals fed the soybean protein diet improved relative to the animals fed the milk protein diet so that at the end of the fifth week of the experiment, the ratios were near unity indicating diet equality. The average values in Table 4 and 6 show that this trend was not entirely due to the continued improvement of the performance of soybean protein fed pigs but also due to a declination of the performance of the milk protein fed pigs. Table 8 summarizes the analyses of variance.

It was concluded from this study that the performance of baby pigs fed the two experimental diets was comparable to the results reported by

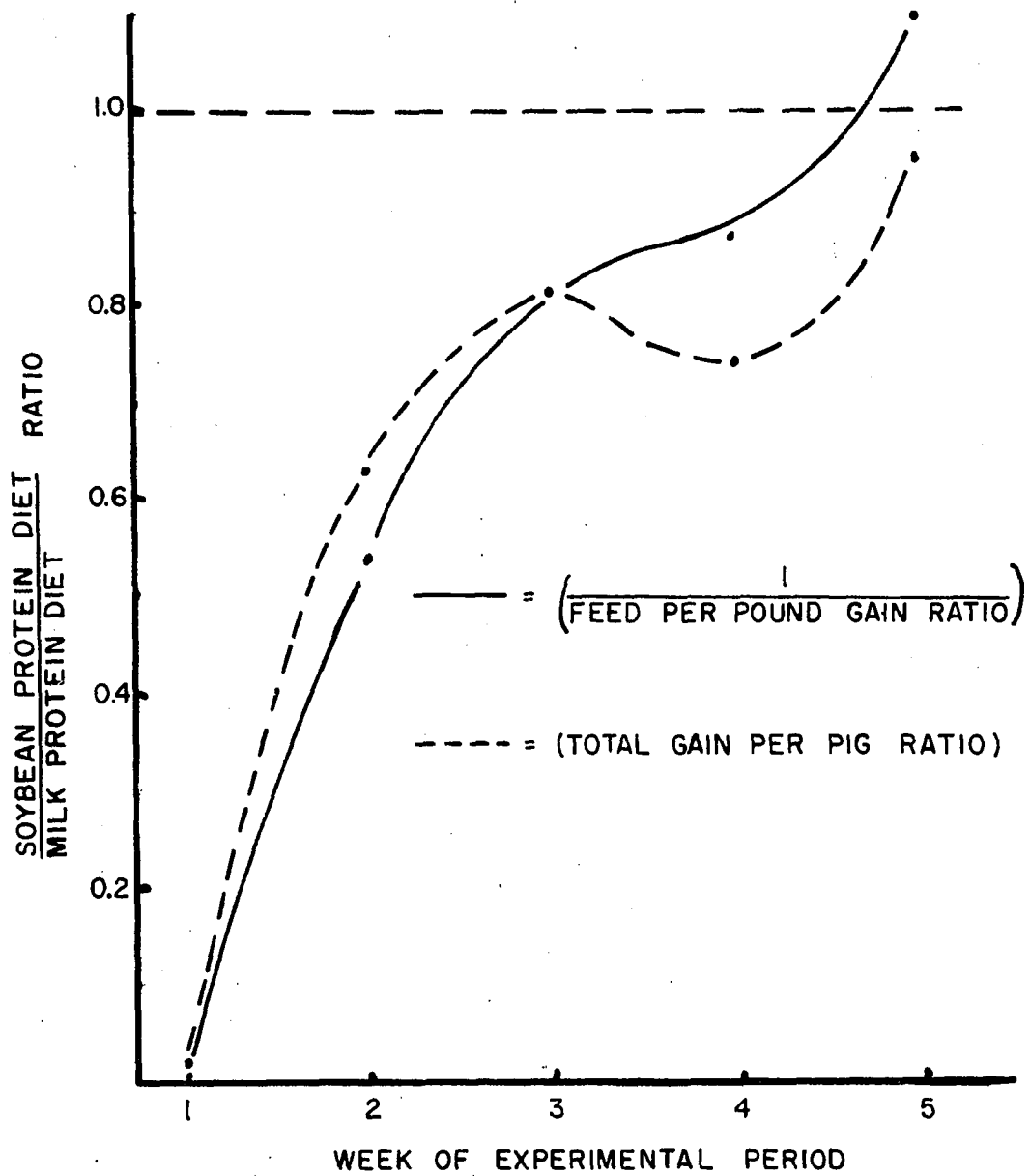


Figure 4. Effect of advancing age on the relative performance ratio of the soybean protein diet to the milk protein diet.  
Experiment 1089

Table 8. Summary of analyses of variance of body weight loss, gains and feed per lb. gain. Experiment 1089

Source	Degrees of freedom	Mean squares		
		Percent body weight loss <sup>a</sup>	Total gain	Feed per lb. gain
Total	9	5.1677	7.5093	0.0359
Treatment	1	36.8257 <sup>b</sup>	47.2193 <sup>b</sup>	.2657 <sup>b</sup>
Replication	4	1.7524	3.9766	.0071
Error	4	.6686	1.1146	.0072

<sup>a</sup>Body weight loss during the terminal 24 hour fast.

<sup>b</sup>Significant ( $P < 0.01$ )

other investigators using similar but less rigidly balanced diets relative to each other. Soybean protein fed pigs lost significantly more weight during the 24 hour fast period, gained significantly less weight and required significantly more feed per pound of gain than pigs fed the milk protein diet. The diet differential decreased from the greatest difference the first week to almost negligible differences during the fifth week of the experiment.

#### Experiment 1026: Preliminary Observations on the Pancreatic Secretion of the Baby Pig

##### Purpose

The purpose of this experiment was to record the secretion rate of the pancreatic juice and to determine the composition of the pancreatic

juice of baby pigs under various experimental conditions.

Also the degree and nature of the variations encountered in the secretion rate and in the composition of the pancreatic juice were investigated to establish the most effective sampling procedure and experimental design to use in subsequent experiments. Pavlov (112) and Grossman et al. (43) express concern about the variations encountered in the rate and composition of pancreatic secretion, as well as others already cited in the Review of Literature.

### Procedure

Pancreatic fistula pigs were prepared for the study of the variations in the rate of secretion of pancreatic juice and the secretion rate was continuously recorded on a kymograph by use of a drop recorder. The feeding activities of the animals were recorded on the same graph by use of a pen "writer" attached to the feeder, mounted by a spring hinge, in such a way that all major feeder movements were recorded on the graph.

Two other unrelated animals were prepared with a pancreatic fistula and the pancreatic juice was collected quantitatively in successive regular intervals and its composition determined. Not every sample of secretion was analyzed for all the separate components or properties of the pancreatic juice because of an insufficient volume of secretion, or difficulty with the analytical procedures.

In both phases of the study the animals were maintained in the collection unit. Detailed notes were kept with emphasis on the time of entrance or exit of the investigator, the time the diets were offered to

the animals, the time the animals started and stopped eating, and the period the animal rested in the presence of the investigator with hopes that these environmental influences could possibly explain the nature of the variation. From the experiences with a few animals prepared in a similar way before this experiment was undertaken, it was felt that there may be a significant association between the time of eating and the pancreatic secretion rate. It seemed evident that the greatest secretion rates were always observed during the time the animal was actually consuming feed and the slowest rates observed while the animal was at rest.

#### Results and discussion

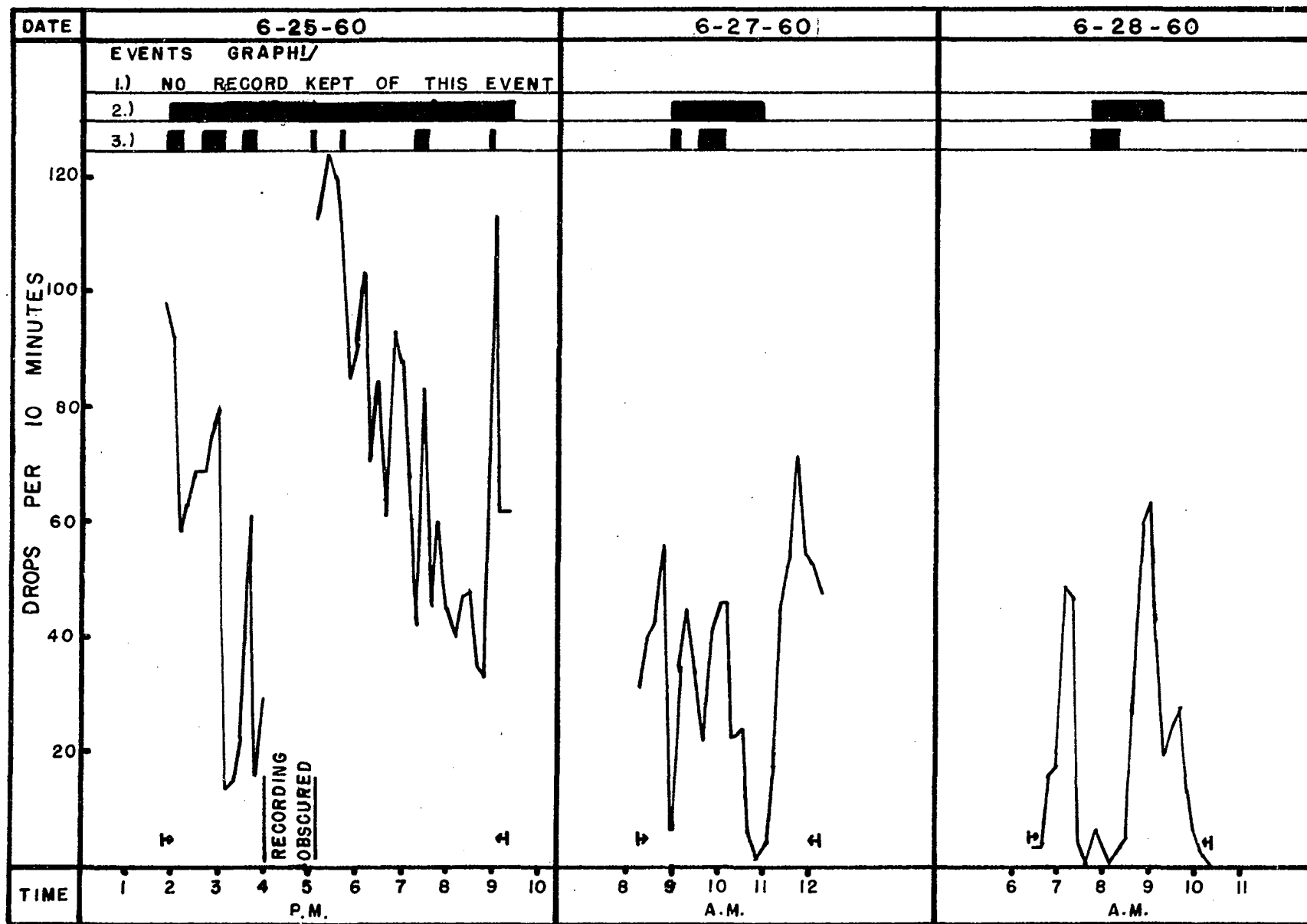
The results of the pancreatic secretion rate recordings will be presented and discussed first and followed by a presentation and discussion of the results relating to the composition of pancreatic juice.

Pancreatic secretion rate recording      The results of the pancreatic secretion rates of two pigs, identified by the numbers 1214 and 1674, are presented and discussed individually.

Recording of Pig 1214      The pancreatic fistula was established in this animal at 43 days of age and the first recording was made the following day. The results of this animal are summarized in Figure 5 which demonstrate that the spontaneous pancreatic secretion rate is extremely variable. From the recordings summarized in Figure 5 an association of the pancreatic secretion rate with eating activity was suggested. However the faster secretion rates are not exclusively associated with food consumption. Due to the persistent variation in

Figure 5. Graphic summary of the pancreatic secretion rate of Pig 1214. Experiment 1026

Explanation: A solid bar in Channel 1 of the "Events Graph" indicates the presence of the investigator in the experimental room, the length of the line indicates the duration of his presence. Likewise, the bar of Channel 2 indicates the availability of feed to the animal and that of Channel 3 indicates the animal's eating activities.



secretion rate it is difficult to assess whether the secretion rate response was manifest simultaneous to the eating activity or after the eating activity had ceased. Between 1 and 4 P.M., June 25, see Figure 5, three peaks in the pancreatic secretion rate curve were exhibited each occurring during or immediately following the three eating sessions indicated in Channel 3 of the "Events Graph". Due to an overlapping of the recording between 4 and 5 P.M. of this same day, the behavior of the secretion rate curve leading to the highest peak recorded for this animal at 5:20 P.M. is not known. The figure indicates that this peak is associated with two eating sessions. Another eating session at 7:15 P.M. was not associated with a secretory response, but a later eating session at 8:50 P.M. was associated with a dramatic secretory response.

Feed was available to this animal at all times, as illustrated in Channel 2 of the "Events Graph" of Figure 5 during June 25. On the contrary feed was offered to the animal only twice daily during June 27 and 28. No recording was made June 26 because it was decided that the animal should be adjusted to this feeding regimen first. The milk protein diet was available throughout the series of recordings shown in Figure 5. Channel 3 of the "Events Graph" of Figure 5 indicates when the animal was eating but it does not illustrate the quantity of diet consumed.

During the course of the 4 hour recording period of the pancreatic secretion rate from this same animal (1214) on June 27 the diet was offered only once and the animal spent two sessions at the feeder consuming the diet. Again the persistent variation in the secretion rate makes it difficult to demonstrate an association between the secretion rate

and eating activity. One major peak immediately preceded the first eating session; another major peak reached its maximum two hours after the beginning of the second eating session. The pancreas was secreting at a substantial rate during this latter eating session. Forty minutes after the close of the second feeding session the secretion rate fell precipitously to a low rate of 1 drop per 10 minutes and this was followed immediately by a marked and rapid rise to the greatest secretion rate recorded on that date.

The following morning (June 28), another 4 hour recording was made and again the diet was offered to the animal only once. The recording on this date demonstrated two peaks in the secretion rate plot. The first peak occurred well in advance of the eating session. The genesis of the second peak coincided with the closure of the eating session, and the peak reached a high rate of 64 drops per 10 minutes 50 minutes later and then fell to 1 drop per 10 minutes after an additional 80 minutes.

Recording of Pig 1674      The second animal to be reported here was a male pig born June 11, 1960. The pancreatic fistula was established in this animal at 43 days of age and recordings of pancreatic secretion rates and eating activity were made on each of the following four days, July 25, 26, 27 and 28.

Due to the erratic behavior of the pancreatic secretion rate recorded from Pig 1214 it was decided that longer recording sessions were needed. The same recording apparatus and methods were used for this animal as were used for Pig 1214, and the diet was also the same. The animal was hand fed daily during July 25, 26 and 27, but was offered the

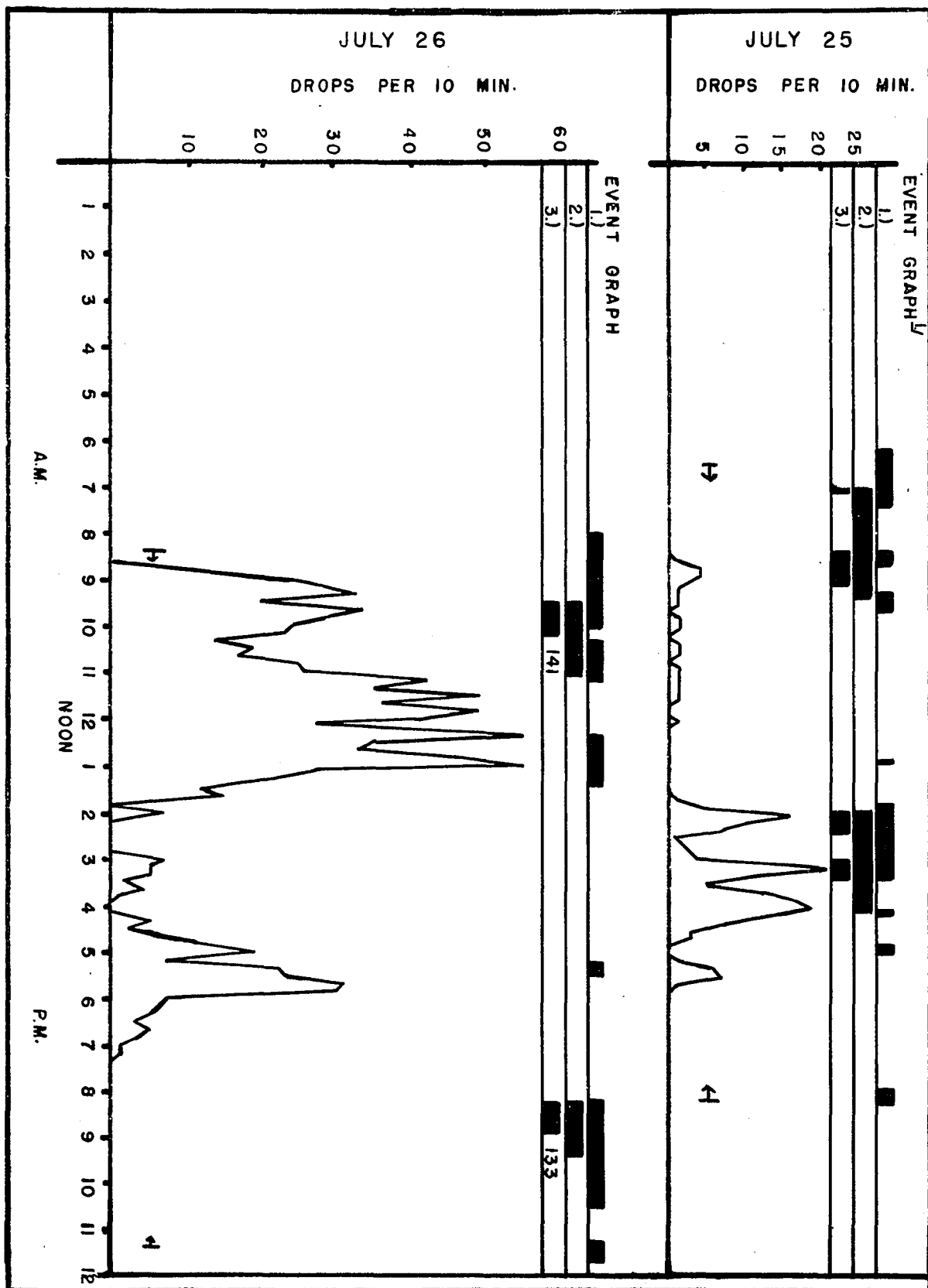
diet continuously during the July 28 recording.

The secretion rates of this animal are summarized in Figure 6. The July 25 recording indicates that a pancreatic secretion response was associated with each of the three eating sessions. From the notes kept on July 25, the animal ate very little feed during the two eating sessions in the morning even though the animal was fasted for a period of 9 hours prior to the morning feeding. At 1:55 P.M. the animal ate vigorously when the feed was presented. The animal ate for about 25 minutes, then rested for 40 minutes and again ate vigorously for a period of 25 minutes. Figure 6 demonstrates a secretion rate response to both of these latter eating sessions, however, two additional responses which were not related to the eating sessions were demonstrated subsequent to the first two responses. Although the quantity of feed consumed at each eating session was not determined, it is known that a total of 248 grams were consumed during the day. According to the notes kept it can be assumed that almost all of this quantity was consumed during the last two sessions. It was noted by the investigator when he entered the room at 8:00 P.M. that the cannula extending from the fistula to the drop recorder lever had been separated apparently by excessive animal movements, thus no recording was obtained at that time. The exact time at which the incident occurred is not known, therefore, it is probable that the complete rest period of the pancreas secretion after 5:50 P.M. was due to this incident.

The recording on July 26 for the same animal revealed that the secretion rates were much greater than those recorded one day previous.

Figure 6. Graphic summary of the pancreatic secretion rate of Pig 1674. Experiment 1026

Explanation: For interpretation of the "Events Graph" see Figure 5, Explanation.



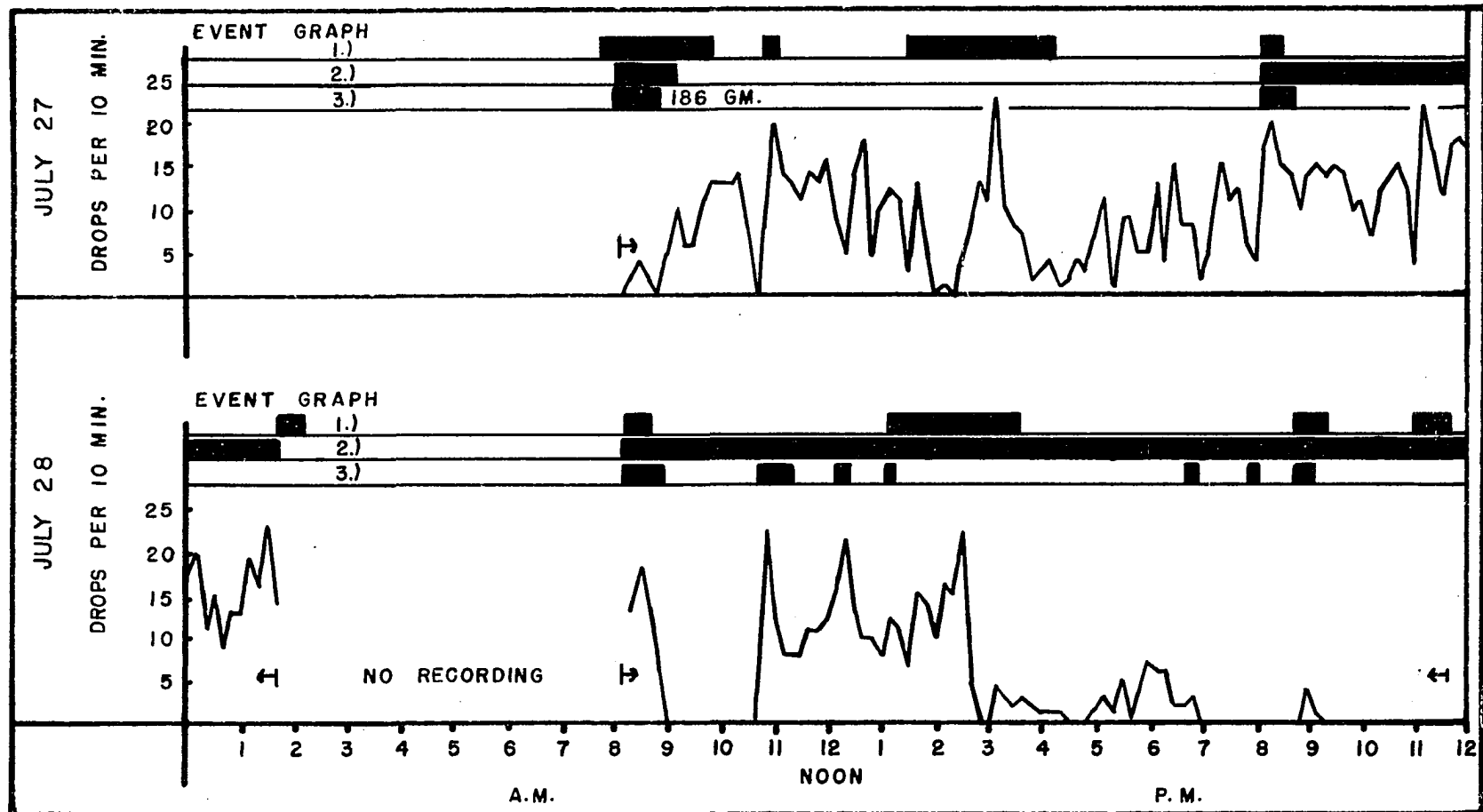


Figure 6. (Continued)

At the time the investigator entered the room in which the experimental animal was maintained there was no pancreatic secretion. The kymograph was started shortly after entrance, and the secretion rate increased greatly a few minutes later. This initial rise occurred well in advance of the presentation of the feed, therefore, it was probably due to the animal's anticipation of being fed. Also the animal was fasted for a period of 18 hours prior to feeding and thus severe hunger could be expected. A continuous decrease in the secretion rate was manifest during the morning feeding. Immediately after the animal stopped eating, the secretion rate increased to about 40 drops per 10 minutes and varied erratically around this mean value for 2 hours after which the rate decreased very suddenly to a complete resting state. The investigator was present in the room at 5:30 P.M. and noted that the animal acted very hungry during his presence. This observation coincides with an isolated peak in the secretion rate plot of Figure 6. The significance of this latter detail may indeed be an important observation. This same animal was fed a second time at 8:16 P.M. and it was observed to eat immediately and very vigorously. The animal ate for about the same length of time and consumed slightly less feed (133 gm vs 141 gm) than it did the previous morning meal. While the secretion rates associated with the morning meal were high no secretion was issued in response to the evening meal. All the recording equipment was checked at that time to insure that it was functional and the fistula and extension cannula tube free of occlusions. It was concluded that the lack of a secretion was in fact due to a resting pancreas gland. The recording of secretion the

following day is positive evidence of a functional gland.

On July 27, another continuous recording was started at 7:55 A.M. and continued to 1:40 A.M. the following day, a period of almost 18 hours. It was noted that there was no secretion when the investigator entered the room at 7:45 A.M. The animal was fed at 8:03 A.M. and the animal proceeded immediately to eat. Secretion was evident about 15 minutes after the animal started eating. The animal consumed 186 gm of the diet in 50 minutes. As demonstrated in Figure 6 the secretion rate continued to rise gradually to an imaginary plateau of about 15 drops per 10 minutes in slightly more than 2 hours. It was noted by the investigator at 10:46 A.M. that the animal managed to turn on its right side in the lying position, causing the cannula to be compressed between the animal's body and the floor of the collection unit. Since the secretion rate increased after the animal was encouraged to change positions by adjusting the harness cords, it was concluded that the decrease in secretion rate to absolute zero at 10:40 A.M. was probably due to occlusion of the fistula extension tube. It is possible however, that the maneuvers of the investigator excited the animal, producing the secretion response. The secretion rate varied around this plateau value for about 3 hours and then gradually decreased toward a resting level, followed by a subsequent increase in rate to 23 drops per 10 minutes and then returning immediately to a near resting rate. This latter isolated peak had a duration of  $1\frac{1}{2}$  hours. The secretion rate following this latter peak increased gradually from a low of 1 drop per 10 minutes to an imaginary plateau of about 13 drops per 10 minutes which had a duration

of almost 6 hours, ending at 1:40 A.M. the following morning (July 28) when the recording was terminated.

Later in the morning of July 28 the last of this series of four recordings was initiated. In the first three recordings the animal was fed twice daily for limited periods. For this last recording it was decided that the diet would be offered to the animal continuously and allow it to eat ad libitum. The recording was started at 8:12 A.M. and continued until 11:30 P.M., a period of little more than 15 hours. The animal had last consumed feed at 8:50 P.M. the previous day and it began to eat immediately when the feed was presented at 8:12 A.M., thus a fast period of almost 12 hours duration preceded the feeding. The animal recorded feeder activity 7 times during the recording. Increased pancreatic secretion rates were recorded in association with the first three of these eating sessions. The latter peak was terminated by a precipitous drop to a rate of zero, followed by only scanty secretion for a period of 4 hours, then no secretion for the rest of the recording except for a very slight response during the last eating session. In reference to the first 3 hours of this recording, the very precipitous drop in the secretion rate during the first eating session was followed by a vertical rise in the secretion rate to the highest value recorded on that date, at a time coincident with the beginning of the second eating session.

#### Discussion of pancreatic secretion rate

Before going into the interpretation of the results it would be well to caution the reader here that the secretion rates were recorded in drops per unit of time and no precautions were taken during this study to measure changes in the size

of drops issued or to measure changes in the viscosity which could effect a change in the drop size. The glass tip from which the drops were formed before dropping onto the recording lever was the same for all recordings presented. For this reason the secretion rates from one day or another or from one pig or the other should be comparable, except for possible changes in the drop size as mentioned above.

Another caution is in order regarding the eating activity recording referred to frequently above. It must be remembered that the animal had to lift and lower the feeder on its hinged mount in order to get the eating activity recording. It is feasible that the animal could maneuver the feeder without in fact consuming the diet, and that the animal could consume a portion of the diet without maneuvering the feeder. However, when the investigator was present in the experimental room, not a single error in the eating activity recording was observed, therefore the recording is considered reliable.

It was noticed with baby pigs studied before these recordings were made that the secretion rate increased while the animals were consuming feed or shortly thereafter. In this study secretion rates were recorded continuously during 25 individual eating sessions of the two animals. Nineteen of the 25 eating sessions (76 percent) were associated with an increase in the secretion rate during or shortly after the time the feed was consumed. Some of these secretion rate response peaks (see Figures 5 and 6) are very prominent and of short duration while other peaks are formed by gradual increases and are of longer duration. Some of these response peaks are essentially a part of an overall secretion response

curve; see Figure 6, plot of secretion rate between 10:40 A.M. and 2:50 P.M. of July 28 as an example. In this figure it is demonstrated that the secretion rate was relatively high during a 4 hour period, yet in this 4 hour period there appears to be three separate response peaks.

Still another interesting relationship seems real from the graphs in Figure 6. Comparing the presence of the investigator (Channel 1, of the "Event Graph" in Figure 6) to the secretion rate plot suggests that the higher secretion rates were frequently recorded during the investigator's presence, however exceptions are also apparent. The plots of secretion rates in Figures 5 and 6 reflect some evidence that the high secretion rate observed at the beginning of most recordings may be related to the entrance of the investigator into the room. This is suggested by the fact that the secretion response curve did in fact start its incline before the feed was presented to the animal. The plot of the secretion rate for July 26 in Figure 6 serves as a good example. Also all three graphs in Figure 5 reflect similar evidence. Although the investigator's presence was not recorded in accumulating the data shown in Figure 5, it is understood that the investigator entered the room shortly before feeding the animal and again when the feeder was removed. Only those visits between feeding are not known. It is conceivable that the initial secretion response, whether it was associated with the investigator's preparing the feed or with the act of consuming the feed or both, is actually the cephalic phase of pancreatic secretion.

A comparison between continuous and twice daily feeding regimens was anticipated and the animals were fed accordingly (see Table 9). It does

Table 9. Summary of pancreatic secretion rates with coefficient of variation. Experiment 1026

Animal No.	Date	Recording period		No. observations	Feeding regimen	Secretion rates (drops/10 min.)			Coefficient of variation
						Range High Low	Mean		
		Hr.	Min.						%
1214	6-25-60	6	40	40	Continuous	124 14	58.59		61.63
1214	6-27-60	4	0	24	Twice daily	71 1	35.38		52.95
1214	6-28-60	4	0	24	Twice daily	64 1	19.08		103.02
1674	7-25-60	13	40	83	Twice daily	21 0	2.45		190.73
1674	7-26-60	14	50	89	Twice daily	55 0	12.63		123.38
1674	7-27-60	17	40	106	Twice daily	23 0	10.03		57.21
1674	7-28-60	15	20	92	Continuous	22 0	4.46		135.13

not appear that any of the expressions of pancreatic secretion rate was affected markedly by the two feeding regimens.

Elman and McCaughn (33) reported that the secretion rate of chronic pancreatic fistula dogs increased steadily from the first day even to the day of death which usually occurred before 8 days. The results obtained with Pig 1214 suggest the opposite result although these results were obtained in a 3 day period and may not accurately indicate the long term trend. The results of Pig 1674 do not suggest a trend in either direction.

Summary and conclusions      Seven individual recording sessions ranging from 4 to 18 hours in duration are summarized in Table 9. The data presented in this table were summarized and calculated on the basis that each 10-minute-period was one observation, this included those 10-minute-periods during which no secretion was recorded. Pancreatic secretion rates were highly variable, the mean secretion rate changed considerably from day to day and from one pig to the other. Note the high degree of variation in secretion rate expressed as the coefficient of variation in Table 9. Although the data resulting from these recordings do not lend themselves to a clear interpretation regarding a possible relationship between eating activity and secretion rate, evidence has been obtained which suggests that the pancreatic secretion rate will frequently increase immediately before or during the act of eating (maybe the cephalic phase) and this response will be of short duration. A more sustained response (possibly the intestinal phase) is often obtained after the animal has completed the eating act. Results obtained

on days of continuous feeding do not markedly differ from the results obtained on those days the animals were fed twice daily. Differences that were observed with one animal are not supported fully by the second and are difficult to explain in terms of feeding schedules.

The results obtained here are not in complete agreement with those reported by Elman and McCaughn (33), Pavlov (112), and Thomas (129), and others, indicating that a chronic pancreatic fistula dog increases its pancreatic juice output steadily during the post-operative period. These data do not clearly dictate a collection period which could be standardized to the feeding schedule to yield a reliable sample of the exocrine pancreatic output. It seems apparent that a collection period of 4 or 6 hours is essential, and that this collection period should start when the diet (test meal) is offered to the animal. The very erratic variation in secretion rate of the pancreas suggests that the pancreas gland is indeed a highly responsive organ; the output of this gland is apparently controlled by a mechanism with considerable oscillatory activity. That these oscillations in pancreatic secretion rate can be reduced by adjusting the feeding schedule or the experimental environment seems unlikely.

Composition of pancreatic juice      In accordance with the objectives listed for this experiment, pancreatic secretion was obtained via pancreatic fistulae from several animals and the total protease, amylase and lipase activities were determined, and the buffering capacity titrated according to the methods already described. Animals were prepared with a pancreatic fistula and the pancreatic juice collected in

pilot experiments before the collection of pancreatic secretion from the two animals to be presented below.

Discussion of results      The data accumulated from two animals (9535 and 9948) are summarized in Tables 10 and 11. Before going into a discussion of the results presented in Tables 10 and 11 it would be well to explain the arrangement and calculations used in setting up these tables. The first column is the sample code; the first character of the code identifies the month, the next character the day of the month, and the last character identifies the sample number of the pancreatic juice collected on that date. The second column of Tables 10 and 11 indicate the duration of each collection period in minutes. Most of the collection periods were exactly 60 minutes in duration with few exceptions. The third column indicates the volume of secretion issued during the collection period, and the fourth column is only to express the secretion rate during all periods on an equivalent time unit (hour) basis. The columns that follow express the composition of the secretion per milliliter of pancreatic juice and the total hourly output of each component. Figures 7 and 8 graphically illustrate these results. Figure 7 represents the data from Pig 9535 and Figure 8 the data from Pig 9948.

Pig 9535 was born on February 16, (2-16) and was 31 days of age on the day of the first collection. The pancreatic fistula was established 4 days before the first collection was started. The animal was observed eating 5 hours after the conclusion of the surgical operation. No secretion was observed until the third day after the operation. The last collection of pancreatic juice from this animal was taken 5 days after the

Table 10. Composition of pancreatic juice collected from Pig 9535. Experiment 1026

Sample code	Collection period	Volume secreted ml.		Buffering capacity (meq Hcl)		Amylase activity (mg. maltose eq.)		Lipase activity (meq. KOH)		
		Minutes	Period	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.
3-19-1	60		0.65	0.65						
3-19-2	60		0.95	0.95						
3-19-3	95		0.05	0.031						
3-19-4	160		1.50	0.564			170.6	96.2	0.063	0.0355
3-19-5	370		9.00	1.46						
3-21-1	60		3.20	3.20	0.949	3.036	130.62	418.0	0.120	0.384
3-21-2	80		2.40	1.80	0.799	1.438	54.36	97.8	0.120	0.216
3-21-3	60		3.00	3.00	0.599	1.797	55.1	165.3	0.033	0.099
3-21-4	60		2.00	2.00	0.399	0.799	61.91	123.8	0.078	0.156
3-21-5	60		1.20	1.20	0.199	0.240				
3-21-6	60		1.90	1.90			100.42	190.8	0.048	0.091
3-24-1	60		9.10	9.10	0.549	4.998	179.69	1635.2	0.153	1.392
3-24-2	60		8.5	8.5	0.599	5.093			0.078	0.663
3-24-3	77		11.2	8.68	0.499	4.334	75.88	658.6	0.078	0.677
3-24-4	60		10.2	10.2	0.449	4.584	199.44	2034.3	0.084	0.857
3-24-5	60		11.0	11.0	0.849	9.337	76.78	844.6	0.120	1.320
3-24-6	60									

Table 11. Composition of pancreatic juice collected from Pig 9948. Experiment 1026

Sample code	Collection period	Volume secreted ml.		Buffering capacity (meq HCl)		Protease activity (mg. Pancreatin eq.)		Amylase activity (mg. maltose eq.)		Lipase activity (meq. KOH)	
	Minutes	Period	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.
3-31-1	60	0.30	0.30			0.241	0.072				
3-31-2	60	0.30	0.30			0.105	0.032				
3-31-3	60	0.20	0.20			0.088	0.018				
3-31-4	60	0.10	0.10			0.013	0.0013				
3-31-5	60	0.20	0.20			0.130	0.026				
3-31-6	60	0.35	0.35			Beyond Std. curve					
3-31-7	60	0.45	0.45			0.60	0.270				
3-31-8	30	0.35	0.70			0.48	0.336				
4-2-1	97	4.0	2.49			0.372	0.926				
4-2-2	63	1.9	1.81								
4-2-3	60	3.7	3.7			0.284	1.051			0.332	1.228
4-2-4	60	5.2	5.2			0.296	1.539			0.344	1.789
4-2-5	69	5.1	4.44			0.352	1.563			0.223	0.990
4-2-6	60	6.0	6.0			0.162	0.972			0.130	0.780
4-5-1	60	7.4	7.4	0.849	6.281			756.4	5597.4	0.390	2.886
4-5-2	60	6.5	6.5	0.898	5.843			718.8	4672.2	0.223	1.450
4-5-3	58	4.9	5.05	0.898	4.539			748.1	3777.9	0.209	1.055
4-5-4	77	12.0	9.38	1.248	11.709			715.4	6710.5	0.191	1.792
4-5-5	60	9.8	9.8	1.049	10.276			740.7	7258.9	0.135	1.323
4-5-6	113	8.3	4.37	0.849	3.710			772.1	3374.1	0.228	0.996
4-5-7	60	7.2	7.2	0.699	5.033			723.9	5212.1	0.269	1.937

Table 11. (Continued)

Sample code	Collection period	Volume secreted ml.		Buffering capacity (meq HCl)		Protease activity (mg. Pancreatin eq.)		Amylase activity (mg. maltose eq.)		Lipase activity (meq. KOH)	
	Minutes	Period	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.	Per ml.	Per hr.
4-7-1	60	4.5	4.5	1.798	8.089			626.5	2819.3	0.111	0.500
4-7-2	60	4.9	4.9	1.897	9.297			660.0	3234.0	0.107	0.524
4-7-3	60	8.4	8.4	1.099	9.227			694.0	5829.6	0.121	1.016
4-7-4	90	7.4	4.94	1.398	6.905			695.0	3433.3	0.125	0.618
4-7-5	60	3.2	3.2	0.699	2.237			721.0	2307.2	0.214	0.685
4-7-6	60	2.1	2.1					724.0	1520.4	0.353	0.741

Figure 7. Composition of pancreatic juice collected from Pig 9535.  
Experiment 1026

Explanation: Day to day variations in the concentration of each component can be visualized by observing the graph of each component across the figure from left to right. The relative change in the concentration of each component with respect to the other components can be visualized by comparing the profiles of the graphs of each component across the figure from top to bottom.

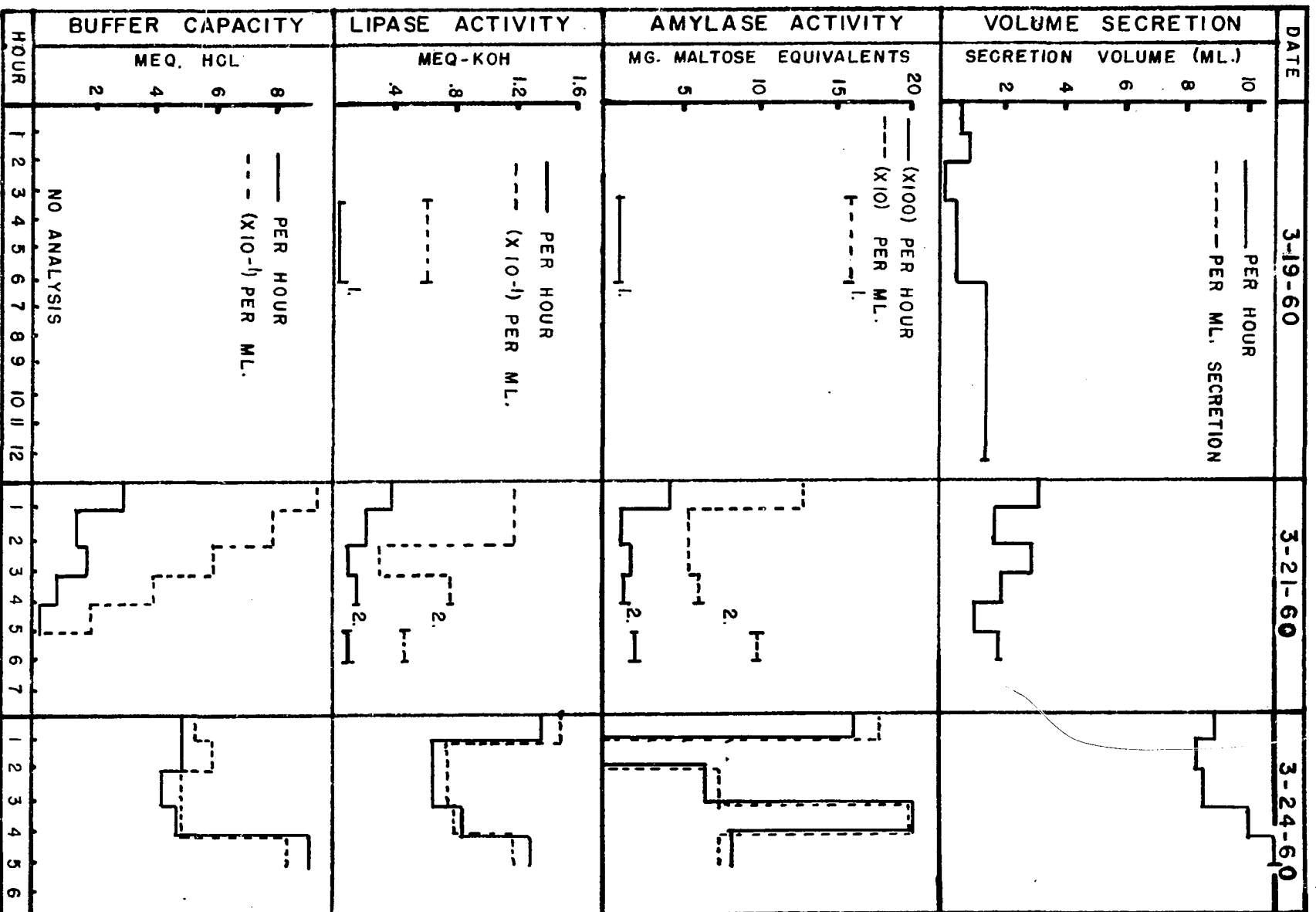
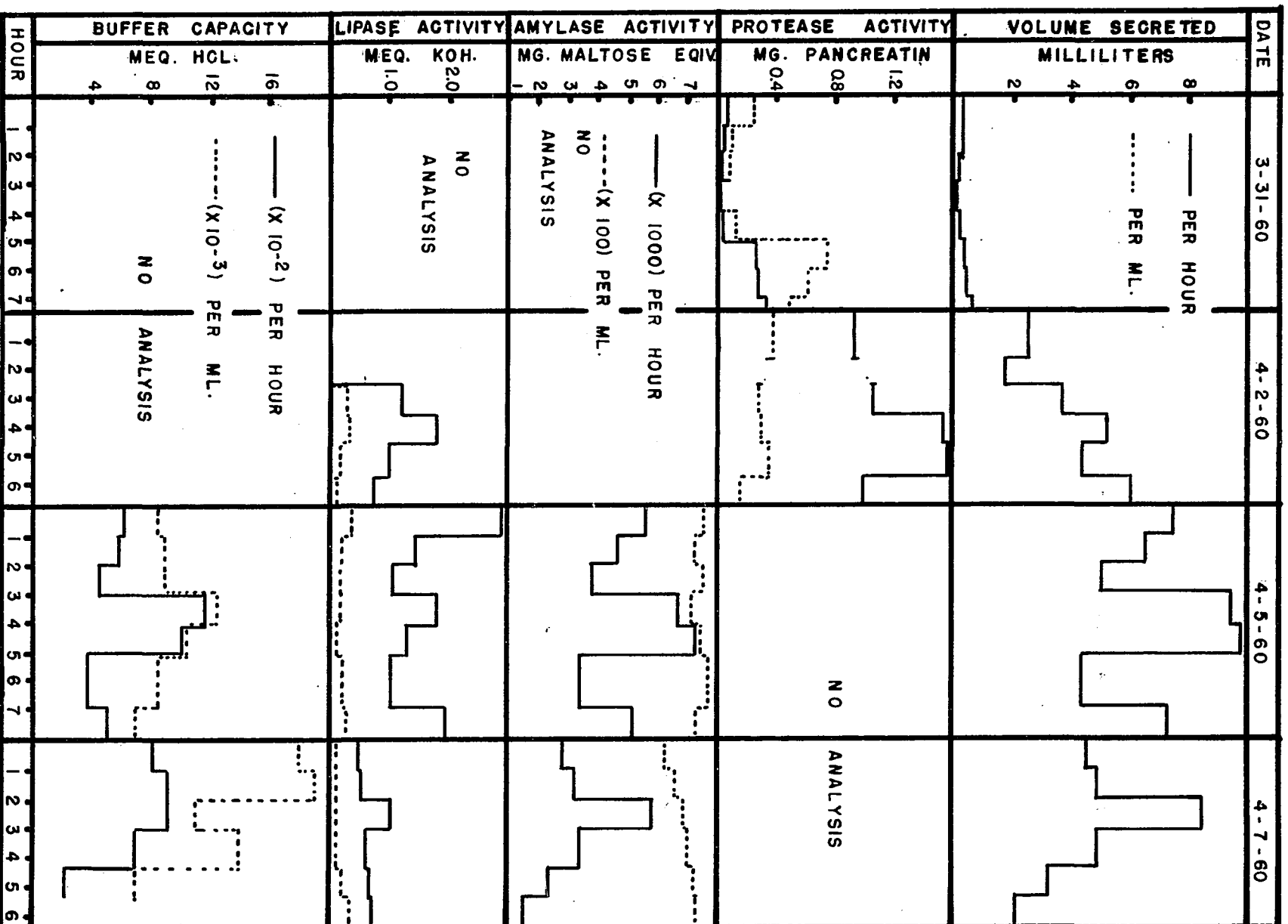


Figure 8. Composition of pancreatic juice collected from Pig 9948.  
Experiment 1026

Explanation: For interpretation of this figure, see Figure 7, Explanation.



first. The animal was fed the experimental soybean protein diet from 3-16 through 3-21, and then switched to the experimental milk protein diet from 3-22 through 3-26. Both diets were offered to the animal continuously. The animal was observed eating during the collection of sample 3-19-3 and during the collection of all samples on 3-21 except sample 3-21-6. On 3-24 the animal was quite inactive with the exception that it was observed eating during the collection of samples 3-24-2 and 3-24-5. The animal consumed 55, 29, and 112 grams of diet on 3-19, 3-21, and 3-24 respectively, however, the amount consumed during the actual collection period was not determined.

The data in Table 10 demonstrates a high degree of variation in secretion rate between samples. A gradual increase in secretion rate seems apparent from the first day to the last day of collection from this animal, however, due to the great variations this trend is questioned. Also the data in this table yield considerable evidence that the concentration of the various components of pancreatic juice are highly variable. It is difficult to visualize any relationship between the per unit volume concentration of the three enzyme activities determined, see Figure 7. Each component seems to vary freely without influence from the other components. This can be interpreted as evidence that the changes in concentration of the pancreatic juice components were not due to simple dilution or concentration of the various components held in a constant ratio to one another. This is easily seen in Figure 7 because all the components are plotted on a common abscissa, thus simultaneous changes in the concentration per milliliter of secretion (broken lines)

are clearly visible. It is possible that the high hourly pancreatic output on 3-24 as compared to 3-19 and 3-21 was due to the larger quantity of feed consumed on that date.

Due to difficulties with the protease method no analysis of the protease activity of the secretion is reported.

The results of the second animal, Pig 9948, are summarized in Table 11 and Figure 8. The pancreatic fistula was established in this animal at 28 days of age and the first pancreatic secretion was collected 2 days later (3-31), followed by subsequent collections on 4-2, 4-5 and 4-7. The pancreas was secreting during the surgical operation. The animal ate actively shortly after recovery from the anesthetic. The animal consumed the following quantities of feed on each of the collection days listed above in chronological order, 43, 30, 106, and 85 grams respectively. The soybean protein diet was offered to the animal continuously from 3-30 through 4-5, and then the animal was switched to the milk protein diet until termination of the study. The animal vomited once (4-1) during the study period.

The animal was not observed eating at all during the collection period on date 3-31, however it was observed eating during the collection of samples 1 and 3 on date 4-2; samples 5, 6, and 7 on date 4-5; and samples 1, 2, 3, 5, and 6 on date 4-7.

The results obtained from this animal are generally in good agreement with the results obtained from Pig 9535.

The volume secretion rate plot, see Figure 8, supports the trend observed previously; that the secretion rate increases gradually each day

after the pancreatic fistula was established. The possibility that this is actually related to the quantity of feed consumed as suggested previously is further supported here.

As was observed with Pig 9535, the concentration of the pancreatic juice components is variable, although not as variable in comparison.

The protease concentration which was not determined for the secretion from Pig 9535 was determined on the secretion issued from this animal during two collection periods, and the results indicate that it is also subject to considerable variation.

In general the amylase and lipase concentration and the buffer capacity were higher for this animal than for Pig 9535. This can be seen by comparing the data summarized in Tables 10 and 11.

Summary and conclusions      In accordance with the major objectives of this study, secretion was collected continuously via pancreatic fistulae from two pigs over periods of 6 to 12 hours at 1 hour intervals (with few exceptions) and analyzed for their digestive enzyme activities and buffering capacity, in an effort to gain some knowledge of its properties and the variations encountered; this knowledge to be applied in establishing a sampling technique which would allow quantitative comparisons from one animal to another in future studies.

Large variations were encountered in hourly pancreatic secretion rates from hour to hour and from day to day. Large variations were also encountered in the composition of pancreatic juice, including protease amylase and lipase activities and the buffering capacity. The concentration of pancreatic juice components varied in a most unorganized

manner. These data do not support a parallel relationship between the various components of the pancreatic juice which could be brought about by simple dilution or concentration of all the components without changing the quantity of one component relative to the other components. There is some evidence that the volume secretion rate may be affected by the total quantity of diet consumed during the day of collection; an increase in feed consumption being associated with a greater secretion rate. Consumption of feed during the actual collection of secretion had only ambiguous effects on the secretion rate.

Due to the variability of the pancreatic secretion rate and of the composition of the pancreatic juice, it is apparent that an accurate evaluation of the digestive functions of the pancreas and dietary influences on the exocrine secretion of the gland will be extremely difficult to attain. From these data it appears that the only factors which have an influence on pancreatic secretion and are subject to experimental control are the total quantity of feed eaten and to a lesser extent the time at which the feed was consumed and the history of the animal's eating activities prior to the collection.

#### Experiment 1074: Development of Pancreatic Digestion of the Baby Pig

##### Purpose

The purpose of this experiment was to compare the digestibility of the two experimental diets on 4-week-old baby pigs with and without pancreatic digestion and to associate the differences in the

digestibility of dry matter and protein with the quantitative output of pancreatic enzymes.

#### Procedure

Four littermate male pigs were weaned from their dam at 18 days of age, fasted for 12 hours and then fed their respective experimental diets ad libitum the remainder of the first 2 post-weaning days. Then the feeding schedule was switched to a limited-twice-daily hand feeding regimen, in an attempt to reduce the variation of the pancreatic secretion in accordance with the results obtained in Experiment 1026. The twice daily hand feeding regimen was started at 20 days of age to allow the animals to condition themselves to the rhythm of this regimen before the pancreatic fistulae were established and the secretion collected. The feed was offered to the animals at a rate of 80 percent of full feed. This was based on the expression that 100 percent of full feed is equal to 0.59 pounds of feed per pound of body weight per animal per week, a value derived from Experiment 942 at the Agricultural and Home Economics Experiment Station, Swine Nutrition Section, Animal Husbandry Department, Iowa State University. It was felt that restricting the animals to a feed intake of 80 percent would cause them to consume all of a given quantity of feed in a short period. By feeding a limited quantity of the diets twice daily the psychic and physiological influences, associated with eating, on the pancreas should also reflect two rhythmic diurnal responses.

Pancreatic fistulae were established on one of the two animals on

each experimental diet at 27 days of age and the other was maintained as a control. The control animals were anesthetized in order that any anesthetic effect would be reflected, but they did not undergo sham operations as the controls in subsequent experiments.

All four animals were fitted with harnesses and placed in the collection unit for collection of feces, urine, and pancreatic juice. See Table 12 for the experimental design and experimental treatment of each animal. Feces and urine were collected at 24 hour intervals and each sample analyzed separately to evaluate and compare the post surgery nitrogen balance status of the control and fistulated animals. Feed consumption was also determined at 24 hour intervals. The animals were sacrificed immediately after termination of the experiment.

### Results and discussion

The results of this experiment are summarized in Table 12. It is appropriate to remark at the onset that one of the objectives of this experiment was not attained because neither of the fistulated animals issued any pancreatic secretion during the entire study. It was assumed that a fibrin clot had possibly blocked the lumen of the cannula or main duct; however, several times a sterile nylon suture was passed into the cannula but no secretion was issued. These results dictate two possibilities which are of vital significance in the further interpretation of the digestibility data. Either the baby pig between the ages of 28 and 35 days does not in fact have an exocrine pancreatic function or that some factor other than an occluded cannula prevented the secretion from being

Table 12. Summary of consumption, excretion, digestibility, biological value and nitrogen retention. Experiment 1074

Diet treatment	Soybean protein diet		Milk protein diet	
Animal number	2150	2158	2156	2157
Pancreatic treatment	Control	Fistula	Control	Fistula
Body wt. day of surgery	9.0	10.25	10.0	11.5
Consumption:				
Total feed, gm.	1,278.	892.	991.	1,171.
Total nitrogen, gm.	39.95	27.88	31.26	36.94
Total dry matter, gm.	1,155.70	806.64	902.90	1,066.90
Excretion:				
Total fecal nitrogen, gm.	9.41	9.67	2.57	12.22
Total urinary nitrogen, gm.	14.50	17.31	10.79	21.29
Total fecal dry matter, gm.	161.25	154.37	69.47	172.35
Nitrogen retention:				
Total, gm.	16.04	0.91	17.90	3.43
Percent of consumption	40.15	3.25	57.26	9.30
Digestibility: (apparent)				
Nitrogen percent	76.44	65.33	91.79	66.93
Dry matter percent	86.05	80.86	92.31	83.85
Biological value (apparent)	52.52	4.98	62.39	13.89

issued through the fistulae of these two animals. If the pancreas does in fact have an appreciable exocrine function, the digestibility data should reflect this and the latter of the two possibilities must be favored. The digestibility data in this case suggest that the pancreas of the control pigs did produce an exocrine secretion with digestive activity, therefore, the following interpretations are presented, assuming that unknown intra-glandular involvements or an occluded cannula prevented the flow of pancreatic juice from the fistula.

Although no pancreatic juice was obtained via the fistula, it was assumed that no pancreatic juice was secreted into the duodenum and therefore the digestibility data are of value in evaluating the effects of excluding pancreatic digestion in baby pigs between 28 and 35 days of age.

It was found during the pre-surgical period that feeding at a rate of 80 percent of full feed was actually more than these animals would consume, thus the feed was offered to all these animals at a rate of 60 percent of full feed (based on the same value representing full feed discussed in the Procedure section of this experiment), during the collection period. Even at this lower rate two animals consumed irregular quantities of the diet and thus the feed consumption varied somewhat between animals as indicated in Table 12. No attempt will be made here to interpret the data for each 24 hour period, because considerable error was introduced due to the irregular eating habits. Therefore the consumption and excretion data were pooled for the 7 day collection period and interpreted accordingly.

The digestibility values for protein and dry matter for the control animals on the soybean protein and milk protein diets agree favorably with results obtained by Hays et al. (55), Neagle (107), Baker (8), and others using more conventional methods. This verifies that the collection unit used in this experiment did not affect the animal's performance. The digestibility of protein by the two pancreatic fistula animals was 65.33 and 66.93 percent for the soybean protein and milk protein diets respectively. These results indicate that the two diets had similar nutritional value when subjected only to gastric and intestinal digestion, and the data further indicate that the improvement in protein and dry matter digestibility in the presence of pancreatic juice was greater for the milk protein diet than for the soybean protein diet.

The nitrogen retention data given in Table 12 reflect differences between the pancreatic fistulated and control animals of a greater magnitude than the digestibility values. It stands to reason that nitrogen absorption and nitrogen retention would be lower for the pancreatic fistulated animals since the digestibility was reduced considerably. This indicates that expressing the nitrogen retention as a percent of the nitrogen absorbed (biological value) would probably be a more meaningful value (see Table 12). It is conceded that the biological value as used here should not be interpreted in its usual way to reflect dietary protein quality in the case of the two pancreatic fistulated animals under surgical nitrogen stress. The total urine nitrogen excretion, total nitrogen retention and biological value data demonstrate that the pancreatic fistulated animals excrete considerably more nitrogen in the urine

than their respective controls in spite of the greatly reduced nitrogen absorption; the overall result is a slight positive nitrogen balance. A serious question was brought forward in regard to this great difference in nitrogen utilization between the pancreatic fistulated animals and the control animals, particularly since the controls did not undergo surgery. A slight depression in nitrogen balance is to be expected following surgery; according to Markowitz et al. (100, p. 75), this negative balance period can be sustained for about 2 weeks following major operations. It is unlikely that the alteration observed in nitrogen utilization can be partitioned accurately into a fraction due to the surgical operation and the remainder due to a lack of exocrine pancreatic secretion. These data indicate that the control animals in subsequent experiments must be subjected to the same surgical stress as the fistulated animals if the results are to yield well defined interpretations.

#### Summary and conclusions

Four littermate animals were studied in this investigation, two were prepared with a pancreatic fistula, the other two were maintained as intact controls. Neither of the fistulated animals issued secretion from the fistula. The digestibility and nitrogen balance data from the fistulated and the control animals were compared and interpreted on the basis that the fistulated animals were without pancreatic digestion whereas the control animals had normal pancreatic digestion. Although no pancreatic juice was issued by either of the two pancreatic fistulated animals, it cannot be taken as unequivocal evidence that there is in fact no exocrine

pancreatic function in the baby pig between 28 and 35 days of age. Control animals, for future experiments in which the pancreas is altered by surgical operation, should undergo identical surgical operations except for the alteration detail itself. Protein and dry matter digestibility were reduced appreciably in the absence of pancreatic secretion into the duodenum in comparison to the controls which was taken as evidence that the pancreas did participate in digestion. Nitrogen retention expressed as a percent of nitrogen consumed or of nitrogen absorbed was markedly reduced in the absence of pancreatic secretion into the duodenum, however the control animals did not undergo surgery and thus this result may be due to an increased loss of nitrogen in the urine of the post-surgical animals.

Experiment 1085: Pancreatic Digestion  
by Baby Pigs 4 and 7 Weeks of Age

Purpose

The purpose of this experiment was essentially the same as that of Experiment 1074. Since neither of the two pancreatic fistulated animals in Experiment 1074 were observed to secrete pancreatic juice via the fistula it was decided to repeat the same basic experimental design, but to include two ages of animals to establish the quantitative output of the gland at two different ages. Also in Experiment 1074 the control animals did not undergo a sham operation and consequently the interpretations were difficult and hypothetical. It was decided that the control animals would undergo the same surgical procedures as the fistulated

animals in this experiment. Protein and dry matter digestibility were to be determined for the sham operated control animals and pancreatic fistulated animals and the results related to the quantitative output of the various digestive enzymes of the pancreas.

### Procedure

Four uniform littermate male pigs from each of two litters were selected. One group of littermate pigs was assigned to the soybean protein diet and the other group to the milk protein diet. Each group of four pigs was divided into two pairs; one pair to be studied at four weeks of age the other at seven weeks of age. One animal from each pair was prepared with a pancreatic fistula whereas the other member of the same pair underwent the sham operation as described in Experimental Methods. This is recognized as a 2x2x2 factorial design with two experimental diets, two pancreas treatments, and two ages, making a total of eight treatments with one animal per treatment. The experimental design can be visualized in Table 13. The pigs were all weaned at 11 days of age and after a 24-hour-fast were offered their respective experimental diets ad libitum. Four days later the feeding schedule was changed to a twice daily hand feeding regimen at 60 percent of full feed (see Experiment 1074 for basis of calculating feed allowance at 60 percent of full feed). Surgery was performed on each animal on the day it reached the age shown in Table 13.

Immediately after the operation, before the animal revived from the anesthetic, the harness was fitted on the animal and the animal placed in

Table 13. Summary of consumption, excretion, digestibility, biological value and nitrogen retention.  
Experiment 1085

Diet treatment	Soybean protein diet				Milk protein diet			
Pancreas treatment	Sham operated Control		Pancreatic Fistula		Sham operated Control		Pancreatic Fistula	
Age date of surgery (days)	26	45	26	45	26	45	26	45
Animal number	4069	4066	4065	4068	4057	4058	4059	4050

Consumption:

Total feed, gm.	654.00	802.00	610.00	774.00	401.00	1,090.00	394.00	1,100.00
Per pound body weight, gm.	70.70	64.16	62.65	58.64	43.35	67.08	41.47	63.77
Total nitrogen, gm.	20.441	25.067	19.066	24.192	12.650	34.384	12.429	34.70
Total dry matter, gm.	591.41	725.25	551.62	699.93	362.62	985.69	356.29	994.73

Excretion:

Total fecal nitrogen, gm.	3.663	3.807	4.873	11.492	0.989	1.201	5.985	3.134
Total urinary nitrogen, gm.	5.394	7.543	4.336	10.297	3.868	11.340	4.275	13.111
Total fecal dry matter, gm.	53.02	85.03	68.73	131.71	17.24	43.27	73.01	60.77

Nitrogen retention:

Total, gm.	11.384	13.717	9.857	2.403	7.793	21.843	2.169	18.455
Percent of consumption	55.69	54.72	51.70	9.93	61.60	63.53	17.45	53.18

Table 13. (Continued)

Diet treatment Pancreas treatment	Soybean protein diet				Milk protein diet			
	Sham operated Control		Pancreatic Fistula		Sham operated Control		Pancreatic Fistula	
Age date of surgery (days)	26	45	26	45	26	45	26	45
Animal number	4069	4066	4065	4068	4057	4058	4059	4050
Digestibility: (apparent)								
Protein, percent	82.08	84.81	74.44	52.50	92.18	96.51	51.85	90.97
Dry matter, percent	91.03	88.28	87.54	81.18	95.25	95.61	79.51	93.89
Biological value: (apparent)	67.85	64.52	69.45	18.92	66.83	65.83	33.86	58.46

the collection unit. Feces and urine were collected for balance and digestibility studies. The collection of feces and urine was started on the fourth post-surgical day and continued for a period of 3 days. The collection was not started until the fourth day following surgery because two animals did not consume their diet as it was anticipated that they would under the feeding regimen employed. The collection period was not continued for more than 3 days because of the possibility that pancreatic involvements may affect the endocrine function of the pancreas. All four animals studied at 45 days of age were taken to the Veterinary Diagnostic Laboratory to investigate this possibility. The animals were observed for gross pathologic lesions and sections of the pancreas were prepared for microscopic evaluation as was described above in the Experimental Methods section of this dissertation.

Four days after surgery pancreatic juice was collected from the two animals prepared with a pancreatic fistula at 45 days of age and the amylase, protease, and lipase activities determined.

### Results and discussion

In this experiment as in Experiment 1074 no pancreatic juice was issued from the two animals prepared with a fistula at 26 days of age. In both of these animals it was noted that the cannula partially filled with secretion during the operation but at no time during the entire post-surgical period was pancreatic secretion apparent. It was assumed that a fibrin clot had formed or that the secretion itself had coagulated thus occluding the fistula. Very small fistula material (0.76 millimeters internal diameter) had to be used due to the very small size of the

pancreatic duct. Passage of a sterile wire into the fistula did not effect a secretion, nor did injection and immediate withdrawal of small quantities of sterile physiological saline. This is not taken as definite proof that there is no exocrine pancreatic secretion before 26 days of age but it offers no contradictory evidence. The two animals prepared with a fistula of the pancreas at 45 days of age were noted to secrete a small quantity of pancreatic juice during the operation and the secretion rate increased soon after the animals revived from the anesthetic and became active.

Due to the lack of pancreatic secretion via the pancreatic fistula of the younger animals the experimental objectives were not fully attained, however the digestibility and nitrogen balance data are of value. As was discussed in Experiment 1074, it can be assumed that no pancreatic juice was secreted into the duodenum of the fistulated animals, thus protein and dry matter digestibility can be compared with or without pancreatic digestion.

The data given in Table 13 demonstrate that the protein digestibility for the soybean protein diet was 82.08 and 84.81 percent for the 4-week-old and 7-week-old sham operated control animals respectively; for the dried skim milk diet the respective digestibility values were 92.18 and 96.51 percent. These values are in good agreement with values obtained using conventional equipment and non-operated animals. Calculation of the percent reduction in protein digestibility caused by the exclusion of pancreatic secretion into the duodenum as compared to the respective sham operated control pigs indicated that the pancreas performs

a more prominent role in the digestion of soybean protein at 7 weeks of age than at 4 weeks and a reverse order of prominence in the digestion of milk protein (see Figure 9). The reduction of protein digestibility at 4 and 7 weeks of age was calculated to be 9.3 and 38.1 percent respectively for the soybean protein diet and 43.8 and 5.7 percent respectively for the milk protein diet. The reduction of dry matter digestibility was 3.8 and 8.0 percent respectively for the soybean protein diet and 16.4 and 1.79 percent respectively for the milk protein diet. Although the reduction in dry matter digestibility was of a smaller magnitude than for protein it yielded the same interpretation as that given above for protein digestibility. It is interesting that the protein and dry matter digestibility for the soybean protein diet was greater than that for the milk protein diet of 4-week-old pigs without pancreatic digestion. It should be recalled here that in Experiment 1074, pigs of a similar age also without pancreatic digestion (prepared with pancreatic fistulae but did not issue secretion), digested the protein and dry matter of the soybean protein diet to the same extent as that of the milk protein diet. In Experiment 1074 the diets were compared on littermate pigs; in this experiment each diet was offered to an unrelated group of littermate pigs and this may explain the discrepancies just discussed.

The exclusion of pancreatic digestion in pigs 7 weeks of age had only slight detrimental effects on protein and dry matter digestibility of the milk protein diet, but had a marked detrimental effect on the protein digestibility and a lesser detrimental effect on the dry matter

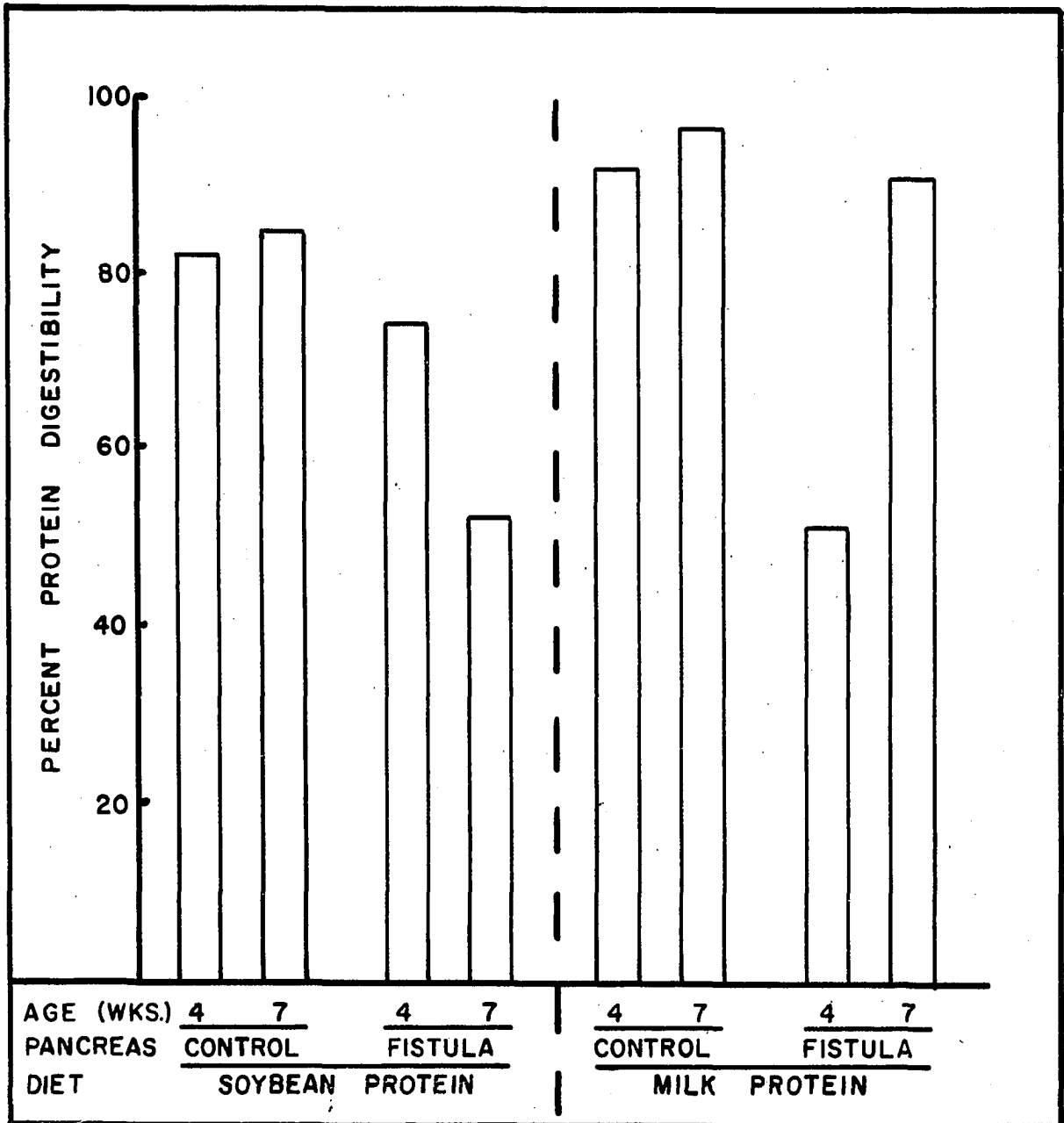


Figure 9. Digestibility of milk and soybean protein by 4- and 7-week-old baby pigs prepared with a pancreatic fistula and their respective sham operated controls. Experiment 1085

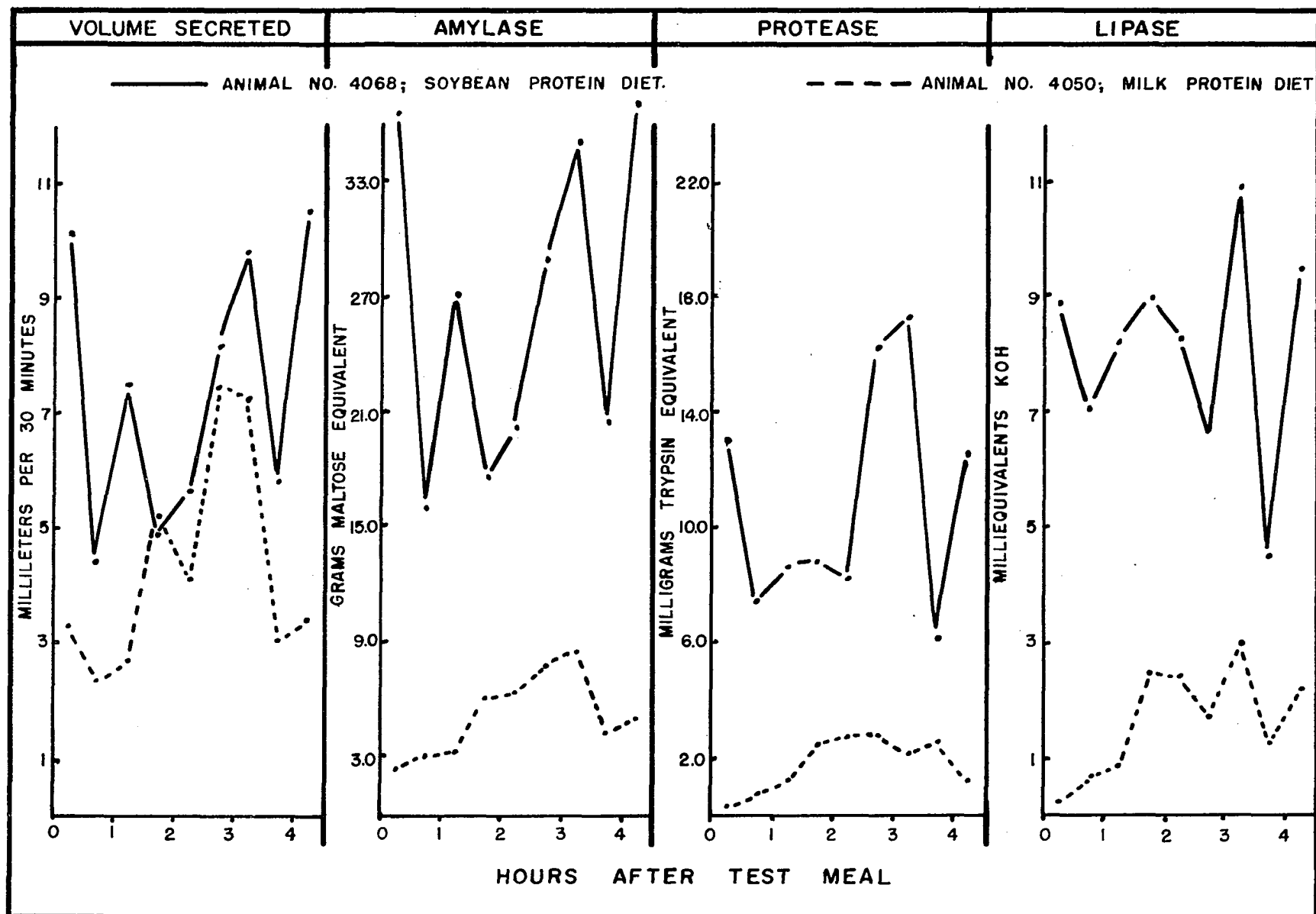
digestibility of the soybean protein diet. Consideration of all the evidence just presented suggests that the pancreas gains a more prominent role in the overall digestive process with advancing age and development of pigs between the ages of 4 and 7 weeks when fed the soybean protein diet but becomes less prominent in the case of pigs fed the milk protein diet.

The volume, and the amylase, protease, and lipase activities of the pancreatic juice collected for a period of  $4\frac{1}{2}$  hours after a single test meal in nine consecutive 30-minute periods are summarized in Table 14 and are graphically illustrated in Figure 10. Neither animal consumed all of the test meal offered in spite of the fact that no feed was available to them for a period of 12 hours before the test meal was offered. Pig 4068 consumed 91 grams of the soybean protein diet during the  $4\frac{1}{2}$  hour collection period; notes maintained during the collection indicated that most of this was consumed during the first, third, and seventh, 30-minute periods. Pig 4050 consumed 173 grams of the milk protein diet during the  $4\frac{1}{2}$  hour collection period; the notes indicated that a considerable quantity was consumed during the fourth 30-minute period, with lesser amounts consumed during the first and seventh 30-minute periods. It was hoped that both of the animals would consume the entire portion of the test meal in the first 30 to 60 minutes after the diet was offered. The similarities in the profiles of the volume secretion rate for the two animals shown in Figure 10 are interesting but of unknown significance due to the irregularity of feed consumption. The data in Table 14 show that the pig fed the soybean protein diet secreted 1.73 times more volume,

Table 14. Summary of the rate of secretion and of the composition of pancreatic juice after a test meal. Experiment 1085

Collection period (minutes)	Volume secreted period (ml)	Amylase		Protease		Lipase	
		Gm. maltose eq. Per ml	Period	Mg. trypsin eq. Per ml	Period	meq. KOH Per ml	Period
Milk protein diet (Animal 4050)							
0-30	3.30	0.711	2.34	0.083	0.27	.1812	.236
30-60	2.35	1.264	2.97	0.300	0.71	.2922	.687
60-90	2.70	1.225	3.31	0.464	1.25	.3204	.865
90-120	5.20	1.147	5.96	0.473	2.46	.4872	2.533
120-150	4.10	1.566	6.42	0.657	2.69	.5988	2.455
150-180	7.40	1.052	7.78	0.382	2.83	.2370	1.754
180-210	7.20	1.186	8.54	0.300	2.16	.4176	3.007
210-240	3.00	1.415	4.25	0.841	2.52	.4176	1.253
240-270	<u>3.40</u>	1.480	<u>5.03</u>	0.366	<u>1.24</u>	.6408	<u>2.179</u>
Total	38.65		46.60		16.13		14.969
Soybean protein diet (Animal 4068)							
0-30	10.10	3.628	36.64	1.292	13.05	.8772	8.860
30-60	4.40	3.632	15.98	1.682	7.40	1.6014	7.046
60-90	7.50	3.594	26.95	1.149	8.62	1.1004	8.253
90-120	4.90	3.610	17.69	1.792	8.78	1.8384	9.008
120-150	5.60	3.610	20.22	1.457	8.16	1.4894	8.343
150-180	8.10	3.573	28.94	2.000	16.20	0.8076	6.542
180-210	9.80	3.582	35.10	1.767	17.32	1.1142	10.919
210-240	5.80	3.539	20.52	1.058	6.14	.7800	4.524
240-270	<u>10.50</u>	3.543	<u>37.20</u>	1.198	<u>12.58</u>	.9054	<u>9.507</u>
Total	66.70		239.24		98.25		73.002
Soybean protein diet/milk protein diet ratio							
	1.73		5.13		6.09		4.88

Figure 10. Changes in the rate of secretion and composition of pancreatic juice of two pigs during a  $4\frac{1}{2}$  hour period after a test meal. Experiment 1085



5.1 times more amylase, 6.1 times more protease, and 4.9 times more lipase during the  $4\frac{1}{2}$  hour collection period than the pig fed the milk protein diet.

The four older animals were examined at the Veterinary Diagnostic Laboratory and it was found the wounds were healing very well of all four animals and there was no evidence of active peritonitis. While the two sham operated control animals had no intestinal adhesions, the two animals with the fistula had numerous adhesions between the small intestine and the polyethylene cannula. The pancreas gland of the two sham operated control animals appeared normal whereas the glands of the fistulated pigs were smaller than normal. The two animals, fistulated and control, fed the soybean protein diet had a rough hair coat and a greasy exudate on the skin which was observed on a pig on the same diet in a previous test. No other gross abnormalities were observed. Histopathological examination of the pancreatic tissue revealed that the tissues of the sham operated control animals were normal. Pancreatic tissue of the fistulated pig which was fed the soybean protein diet, Animal 4068, showed mild fibrosis and atrophy which was most severe in the immediate region of the cannulated duct and negligible in the distal portions of the gland. The epithelium of the intercalated ducts in the region of fibrosis exhibited mild metaplasia and hyperplasia. The islets of Langerhans were difficult to distinguish.

Generally, the pancreatic tissue of the milk protein diet fed pig with a pancreatic fistula, Animal 4050, showed the same microscopic alterations but more intense. The lumen of the pancreatic duct was

partially occluded by the inflammatory reaction and tissue damage caused by the cannula and the islets of Langerhans were not distinguishable. The results of the histopathological examinations indicate that the polyethylene cannula was irritating to the tissue.

It is probable that the lesser output of pancreatic juice and enzymes by Animal 4050 as compared to 4068 could be explained by the more extensive tissue damage. The irregular eating habits exhibited during the collection period and the histological alterations encountered, do not warrant a conclusive interpretation regarding the influences of the two diets on the pancreatic secretion. If it is assumed for the sake of comparison that the data obtained regarding the output of pancreatic juice are truly representative of the normally functioning pancreas of pigs on the two diets, then the secretion results support the results of the protein digestibility studies, namely that the pancreas gains a more prominent role in the overall digestive process for the soybean protein diet fed pigs than pigs fed the milk protein diet. The results of pancreatic output are not easy to interpret due to questionable influences by the two experimental diets, the irregular eating habits and the histological alterations encountered only 7 days post-surgery. These limitations point toward the conclusion that unreasonable risk is associated with the successful accomplishment of the experimental design and objectives. Perhaps it is unreasonable to expect the young pig, accustomed to very frequent consumption of food, to adapt itself to two single feedings daily. The pancreas, recognized as a very sensitive tissue to surgical procedures in the adult, is probably more reactive

in the juvenile gland still in the process of development. Although the results of the digestibility studies in Experiment 1074 and of the 4-week-old group of animals in this experiment are not in perfect agreement, they do suggest that the pancreas does play an appreciable role at this early age and further that the relative role of the pancreas in the digestive processes of the baby pig at various ages could be studied experimentally by the exclusion of pancreatic digestion.

It is possible that the losses of pancreatic juice by the two pancreatic fistula pigs at 45 days of age had an influence on the function of the other digestive glands. Hansson (49) has obtained evidence that plasma amino acids are concentrated in the tissues of the pancreas at a greater rate than other digestive organs. It has been mentioned before that the pancreas tends to hypersecrete shortly after the external fistula is established. These two phenomenon could conceivably put the chronic pancreatic fistula animals under nitrogen stress which could alter the functions of other digestive organs which would not be reflected by the sham operated control pigs.

Experiment 1099: A Comparison of Nutrient  
Digestibility of Pancreatic Duct Ligated vs. Sham  
Operated Control Baby Pigs 4 and 8 Weeks of Age

Purpose

The differences observed in the digestibility of protein and dry matter between baby pigs prepared with a pancreatic fistula and the control animals in Experiments 1074 and 1085 indicated that the exclusion of pancreatic juice and measurement of the reduction of protein and dry

matter digestibility as compared to sham operated control animals would yield indirect evidence to establish the role of pancreatic digestion of baby pigs at various ages. The purpose of this experiment was to determine protein and dry matter digestibility after exclusion of pancreatic digestion of a larger number of baby pigs than could be accommodated by use of the partial restraining collection unit and to compare the results to sham operated control pigs. It was decided that the effect of excluding pancreatic digestion would be evaluated with baby pigs at 4 and 8 weeks of age.

#### Procedure

Four uniform littermate baby pigs were selected from each of four litters. Two of the four groups of littermate pigs were selected for experimental investigation at the age of 4 weeks and they were weaned at the age of 21 days. All the animals were placed in individual digestibility stalls and their respective experimental diets were offered to them ad libitum until 24 hours before the time of the surgical operation. Pancreatic digestion was excluded by ligating the pancreatic duct. The method used for ligating the pancreatic duct and for the preparation of the sham operated control animals was discussed in the Experimental Methods section of this dissertation. The method of collection of feces and urine for this experiment was also discussed in the same section.

A 2x2x2 factorial design was used, consisting of two diets, two ages, and two pancreatic treatments. The experimental design was replicated twice and the data were analyzed by the methods described by

Snedecor (124). See Table 15 for experimental design and arrangement of treatments. The four littermate animals were assigned so that one represented each of the four treatment subclasses (two diets x two pancreatic treatments) within their respective age group. The group of animals studied at 25 days of age were prepared surgically at 20 days of age. Feces and urine collections were initiated 5 days after the surgical operation and were continued for 4 days. The group of animals studied at 56 days of age were prepared surgically at 55 days of age and the feces and urine were collected for a period of 6 days starting 1 day after the operation. After termination of the collection period, all the animals from both age groups were taken to the Veterinary Diagnostic Laboratory for gross and histopathological observation.

### Results and discussion

Consumption, excretion, digestibility, nitrogen retention, and biological value data are summarized in Table 15. The data in this table show that the consumption of dry matter and nitrogen were similar for each of the four treatments within the two age groups. Protein digestibility was significantly higher for the milk protein diet than the soybean protein diet, was significantly reduced by pancreatic duct ligation, and was significantly higher at 56 days than at 26 days of age. See Table 16 and Figure 11. A significant age x dietary protein interaction was also manifested due to the higher digestibility of the soybean protein at 56 days of age as compared to that at 26 days of age while the digestibility of milk protein remained unchanged. The percent of nitrogen retained

Table 15. Summary of consumption, excretion, digestibility, biological value, and nitrogen retention.<sup>a</sup> Experiment 1099

Parameter	Age (weeks)	Soybean protein diet		Milk protein diet	
		SOC <sup>b</sup>	PDL <sup>c</sup>	SOC	PDL
Number of animals	4	2	2	2	2
	8	2	2	2	2
Dry matter consumption, gm.	4	976.20	965.70	968.21	936.64
	8	4114.73	4849.50	4241.17	4777.90
Nitrogen consumption, gm.	4	34.30	33.94	34.68	33.55
	8	144.58	170.40	151.89	171.11
Total fecal nitrogen, gm.	4	8.46	19.96	3.50	7.66
	8	16.84	48.36	6.53	46.77
Total urinary nitrogen, gm.	4	6.99	4.41	4.81	9.04
	8	46.22	44.04	57.87	36.49
Total fecal dry matter, gm.	4	135.50	244.50	70.50	110.50
	8	322.50	655.50	205.50	788.50
Dry matter digestibility, percent	4	84.11	74.42	92.71	88.37
	8	92.04	86.63	95.17	84.02
Protein digestibility, percent	4	71.42	38.86	89.89	77.16
	8	88.12	72.04	95.72	73.38
Nitrogen retention, percent	4	50.30	26.27	76.12	49.49
	8	56.08	46.04	57.68	52.45
Biological value (apparent)	4	69.80	66.57	84.70	64.12
	8	63.58	63.91	60.25	70.55

<sup>a</sup>All values are averages of the values of two animals of each treatment.

<sup>b</sup>Sham operated control, symbolized by SOC.

<sup>c</sup>Pancreatic duct ligated, symbolized by PDL.

Table 16. Summary of analyses of variance of apparent protein and dry matter digestibility, biological value, and nitrogen retention. Experiment 1099

Source	Degrees freedom	Protein digestibility	Mean squares		Biological value
			Dry matter digestibility	Nitrogen retention	
Total	15	320.5971	47.9303	227.0174	75.3923
Replication	1	1.8564	1.3053	24.9001	34.0764
Treatment	7	615.0887 <sup>a</sup>	86.3097 <sup>b</sup>	381.9960 <sup>b</sup>	105.5183
Milk vs soybean (protein)	1	1079.6153 <sup>a</sup>	133.1139 <sup>b</sup>	813.6756 <sup>b</sup>	54.4274
25 vs 56 days (age)	1	674.0514 <sup>b</sup>	83.4025	25.2506	167.7672
SOC vs PDL (operation)	1	1752.0503 <sup>a</sup>	234.0135 <sup>a</sup>	1086.3616 <sup>b</sup>	37.0576
Age X operation	1	11.7477	1.6066	313.1130	279.6421
Operation X protein	1	46.0023	.0370	1.2210	10.1602
Age X protein	1	572.0468 <sup>b</sup>	121.2752 <sup>b</sup>	420.6602	16.5853
Age X operation	1	170.1069	30.7193	13.6902	172.9881
Error	7	71.6400	16.2116	100.9127	51.1686

<sup>a</sup>Significant ( $P < 0.01$ )

<sup>b</sup>Significant ( $P < 0.05$ )

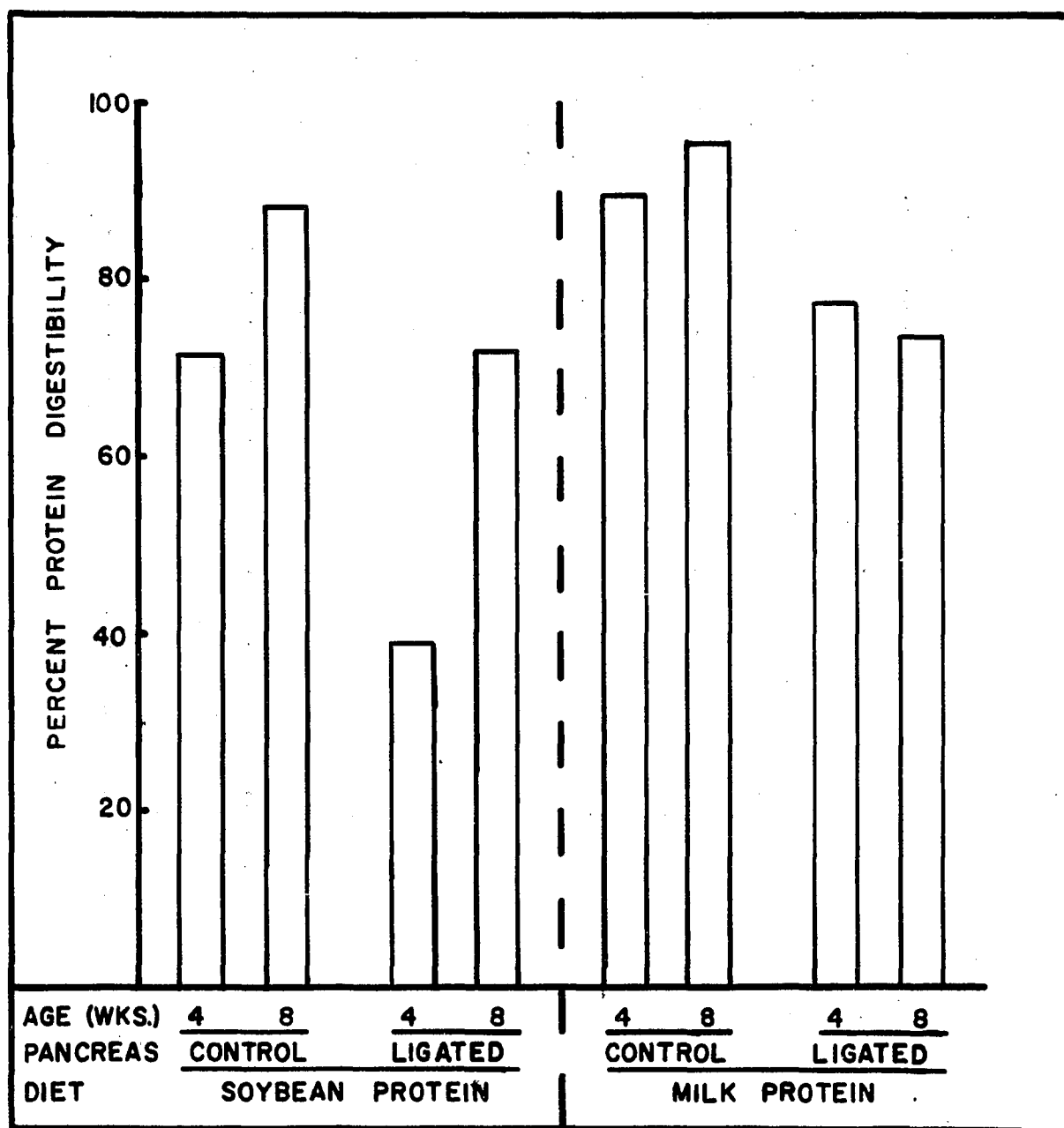


Figure 11. Digestibility of milk and soybean protein by 4- and 8-week-old pigs with ligated pancreatic ducts and their respective sham operated controls. Experiment 1099.

was significantly lower for pigs fed the soybean protein diet as compared to the pigs fed the milk protein diet and the percent of nitrogen retained was significantly reduced by pancreatic duct ligation. Biological value of the dietary proteins was not significantly affected by any of the treatments. However the biological value of the protein for pigs fed the milk protein diet dropped appreciably from a value of 84.7 at 25 days of age to a value of 60.3 at 56 days of age. Hays et al. (55) reported a similar result. The results of this experiment do not support the indication in Experiments 1074 and 1085 that the pancreas gained a more prominent role in the digestion of soybean protein and a less prominent role in the digestion of milk protein as the animals grew older. In fact these results suggest the reverse order of prominence in the case of soybean protein, with relatively small changes in the case of milk protein.

Gross observation of the animal carcasses and their viscera revealed that the wounds were healing nicely with occasional edema at the surgical site. The pancreas glands of the sham operated control animals appeared normal and few peritoneal adhesions were apparent. Gross observation of the pigs with ligated pancreatic ducts showed very similar involvements as the sham operated controls except that the pancreas was atrophied. The lymphatics were distended and the lymph nodes edematous in those animals showing considerable peritoneal adhesions and it was proposed that these involvements were the result of stasis of the lymphatic flow due to the reduced peristalsis associated with intestinal adhesions. Petechial hemorrhages were widely dispersed throughout the carcass but these were thought to be due to the electric euthanasia. Bacterial

examination of the tissues was negative, with one exception where a Streptococcus species was isolated from a surgical abscess.

Histopathological examination of the pancreatic tissues revealed extensive atrophy and fibrosis of all the tissues and dilation of the pancreatic ducts of the pancreatic duct ligated pigs. Lymphocytic infiltration of the pancreas and/or lamina propria of the small intestine were observed in all the pigs regardless of the pancreas treatment except two sham operated control animals. Myelogenic emboli of unknown origin were apparent in the pulmonary arteries of two of the sham operated control animals. The report from the Veterinary Diagnostic Laboratory further indicated that the islets of Langerhans were more prominent in the 56-day-old pigs than the 25-day-old pigs and that the islets of Langerhans were undergoing hydropic degeneration in all four of the 56-day-old pigs with ligated pancreatic ducts.

Experiment 1114: A Comparison of Nutrient Digestibility  
of Pancreatic Duct Ligated vs. Sham Operated Control  
Baby Pigs at 2, 4, and 6 Weeks of Age

Purpose

In Experiment 1099 it was found that ligation of the pancreatic duct of baby pigs fed either the soybean or the milk protein diet caused a significant reduction in protein and dry matter digestibility. However, the relative degree of reduction at 4 and 8 weeks of age was not in agreement with the results obtained in Experiments 1074 and 1085 where pancreatic fistula were used but no secretion was issued. From evidence presented in the Review of Literature and from Experiment 1089, the

growth and efficiency of feed utilization by baby pigs fed a soybean basal diet improves most rapidly between the ages of 2 and 6 weeks. It was decided that the same experimental design as that employed in Experiment 1099 would be used in this experiment to investigate the effects of excluding pancreatic digestion from baby pigs at 2, 4, and 6 weeks of age.

### Procedure

Forty-eight baby pigs were weaned from their dams at 7 days of age. After a 12 hour fast the respective experimental diets were offered to them. All the animals were maintained in floor pens until 5 days before surgery when they were placed in individual digestibility stalls. A 2x2x3 factorial design was employed, consisting of the two experimental diets, two pancreas treatments, and three ages. The basic design was replicated four times. See Table 17 for experimental design and arrangement of treatments. All animals were assigned to their respective pens by completely randomized procedures. The surgery was performed 24 hours before the feces and urine collections were initiated. The method of collection was discussed in the Experimental Methods section of this dissertation. A 7 day collection period was used for the 2-week-old animals due to the small quantities of excreta obtained after 5 days of collection. A 5 day collection period was used for the 4- and 6-week-old pigs. All 16 of the 2-week-old pigs were taken to the Veterinary Diagnostic Laboratory for observation. The 4- and 6-week-old pigs were not taken to the diagnostic laboratory since animals of comparable age were examined in Experiment 1099.

Table 17. Summary of consumption, excretion, digestibility, biological value, and nitrogen retention.<sup>a</sup> Experiment 1114

Parameter	Age (weeks)	Soybean protein diet		Milk protein diet	
		SOC <sup>b</sup>	PDL <sup>c</sup>	SOC	PDL
Number of animals	2	4	3	2	4
	4	4	3	4	4
	6	4	4	3	3
Dietary dry matter chromium equivalent in feces, gm.	2	608.4	413.2	347.8	368.6
	4	619.8	519.4	690.5	567.2
	6	958.4	1163.4	1344.8	1159.5
Dietary nitrogen chromium equivalent in feces, gm.	2	20.43	10.72	11.82	12.54
	4	20.81	17.44	23.49	19.30
	6	32.26	39.06	45.75	39.44
Total fecal nitrogen, gm.	2	6.67	7.71	1.77	7.28
	4	6.33	11.99	3.04	9.15
	6	8.44	21.13	4.88	17.12

<sup>a</sup>All values are averages of the individual values calculated from the pigs of each treatment.

<sup>b</sup>Sham operated control, symbolized by SOC.

<sup>c</sup>Pancreatic duct ligated, symbolized by PDL.

Table 17. (Continued)

Parameter	Age (weeks)	Soybean protein diet		Milk protein diet	
		SOC <sup>b</sup>	PDL <sup>c</sup>	SOC	PDL
Total urinary nitrogen, gm.	2	10.78	5.30	4.11	7.15
	4	9.36	6.03	11.12	8.26
	6	11.48	15.09	30.45	16.66
Total fecal dry matter, gm.	2	112.9	99.5	44.0	92.8
	4	110.2	167.2	77.9	127.6
	6	144.6	285.5	131.8	235.5
Protein digestibility, percent	2	65.64	30.38	68.27	55.80
	4	69.23	29.92	88.16	53.48
	6	74.58	46.18	89.22	59.05
Dry matter digestibility, percent	2	80.62	67.89	79.60	73.72
	4	82.06	67.24	88.22	77.25
	6	85.36	75.58	90.12	80.56
Nitrogen retention, percent	2	12.49	-12.79	2.74	-17.28
	4	23.50	-5.85	31.34	12.49
	6	35.34	7.12	23.08	13.03
Biological value (apparent)	2	17.55	-63.03	-29.76	-135.65
	4	33.15	-56.54	36.09	14.04
	6	47.69	15.63	25.98	23.89

### Results and discussion

The results of this experiment are summarized in Table 17. It can be seen from the table that there were five missing values in the experimental design. This is because three of the 2-week-old pigs did not excrete any feces containing the chromium oxide and two of the 6-week-old pigs were removed from the experiment because they were very unhealthy. The quality of the 48 animals selected for this study generally was poor in comparison to the uniformity and vigor of the animals used in Experiment 1099. The data accumulated in this experiment were analyzed by the approximate method of analysis of variance for disproportionate subclass numbers in a factorial design; see Snedecor (124, p. 385). A summary of the analyses of variance is given in Table 18. There were no significant treatment or interaction effects for the percent nitrogen retention or biological value. The data in Table 17 indicate that this is because of the extreme variations between the four animals within a single experimental cell. The average value for each of the cells does suggest that both the biological value and nitrogen retention are generally reduced by ligation of the pancreatic duct. Again in this experiment as in Experiment 1074, 1085, and 1099 protein digestibility was affected appreciably. Although dry matter digestibility was affected in a similar manner as the protein digestibility, the effect was of a lower magnitude. There was a significant difference in protein digestibility between the three age groups of animals. The data indicate that the digestibility increased for both diets each subsequent 2 week period. It should be emphasized here that two of the four sham operated control

Table 18. Summary of analyses of variance of apparent protein and dry matter digestibility, biological value, and nitrogen retention. Experiment 1114

Source	Degrees freedom	Protein digestibility	Mean squares		Biological value
			Dry matter digestibility	Nitrogen retention	
Total	42				
Treatment	11	1279.85 <sup>a</sup>	179.14	960.45	10,272.52
Age	2	493.01 <sup>b</sup>	185.78 <sup>a</sup>	2076.24	23,585.49
Protein	1	2361.38 <sup>a</sup>	214.20 <sup>b</sup>	4.55	1,517.02
Operation	1	9421.82 <sup>a</sup>	1170.22 <sup>a</sup>	5140.69	32,725.59
Age X protein	2	56.96	26.41	397.22	8,797.72
Age X operation	2	129.13	11.72	13.51	5,380.39
Protein X operation	1	650.58 <sup>b</sup>	114.49	359.44	690.25
Age X protein X operation	2	142.82	11.90	43.18	1,171.30
Error	31	107.51 <sup>c</sup>	34.47	116,790.09	9,646.03

<sup>a</sup>Significant ( $P < 0.01$ ).

<sup>b</sup>Significant ( $P < 0.05$ ).

<sup>c</sup>Has 30 degrees of freedom.

pigs on the milk protein diet at 2 weeks of age did not eat and consequently no digestibility values were obtained. A third animal from the same group ate a very small quantity of feed and the protein digestibility value is very low in comparison to values calculated in other studies. It is probable that the fourth value is most representative.

Milk protein was found to be significantly more digestible than soybean protein and the digestibility of both proteins was significantly reduced by ligation of the pancreatic duct. Also there was a significant interaction between the dietary protein source and the pancreas treatment. The data indicate that this is due to the greater reduction of protein digestibility by ligation of the pancreatic duct of pigs fed the soybean protein diet than pigs fed the milk protein diet. However, there was no significant interaction between age and dietary protein or between age and pancreas treatment. These results agree well with the results of Experiment 1099. These results support the proposition that the pancreas plays a more prominent role in the digestion of soybean protein than in the digestion of milk protein. However, these data do not support that the pancreas gains a more prominent role with advancing age in the case of soybean protein fed pigs and a decreasing role in the case of milk protein fed pigs. The major experimental influences on protein digestibility can be visualized in Figure 12.

Dry matter digestibility values reflect the same experimental influences as were just described for protein digestibility. Dry matter digestibility increased significantly with age, was significantly higher for milk protein fed pigs than soybean protein fed pigs and was

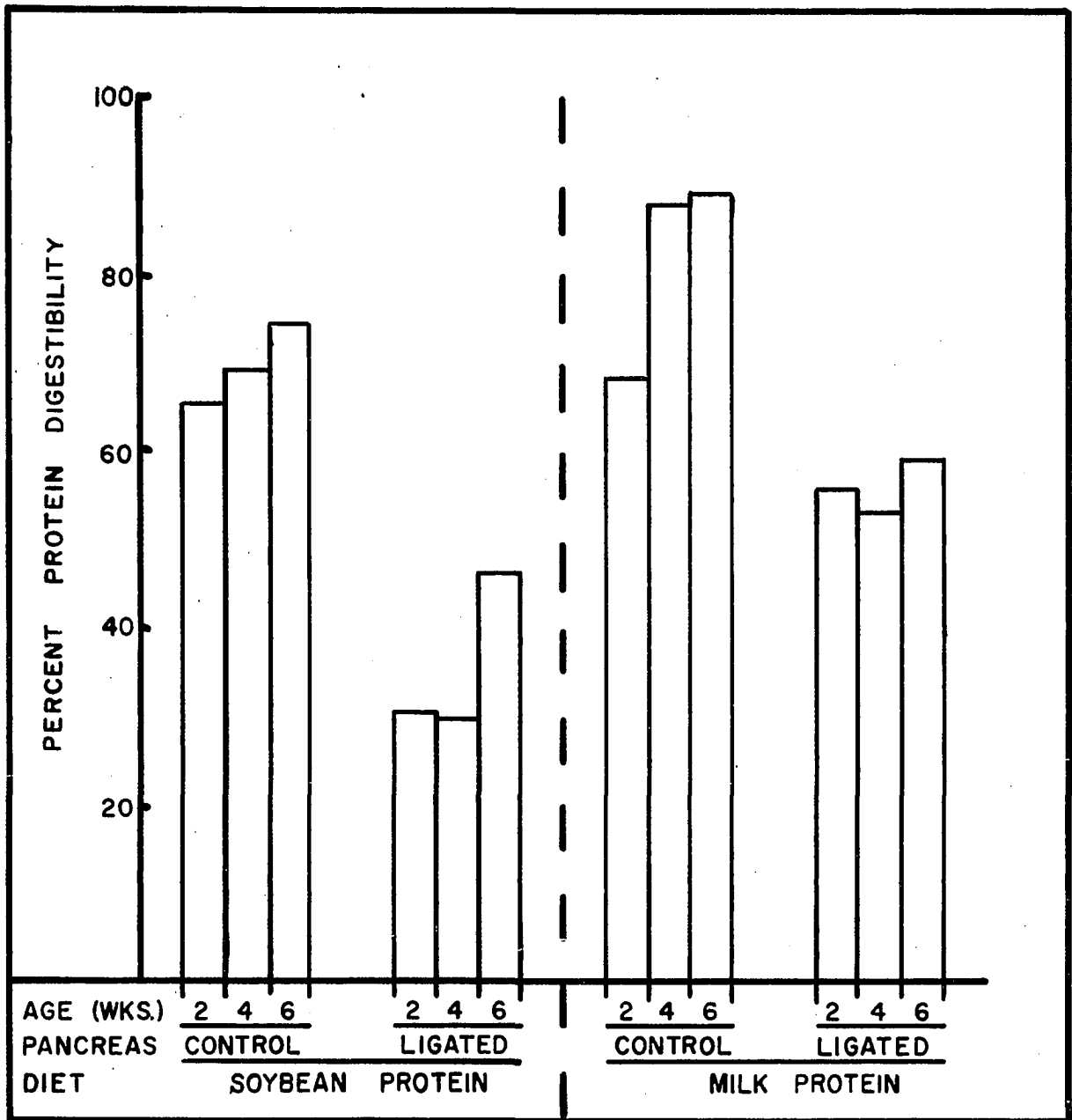


Figure 12. Digestibility of milk and soybean protein by 2-, 4-, and 6-week old pigs with ligated pancreatic ducts and their respective sham operated controls. Experiment 1114

significantly reduced by ligation of the pancreatic ducts. A summary of the analyses of variance is presented in Table 18.

The report of the gross and histopathological examination of the 16 2-week-old baby pigs indicated that the pancreas was severely affected by ligation of the pancreatic duct. The islets of Langerhans were not observed in 14 of the 16 animals with only few immature islets present in the remaining two animals. All the pigs with ligated pancreatic ducts revealed severe atrophy and fibrosis of the pancreas tissue, the pancreatic ducts were dilated and only few acini were observed. The pancreas of three of these animals were infiltrated with lymphocytic cells. With the exception of Animal 7261S, all the sham operated control animals had normal appearing pancreas glands. The pancreatic tissue from Animal 7261S resembled the tissues of pancreatic duct ligated animals very closely. Perhaps this condition resulted from extensive trauma to the gland during the surgical procedures. This finding is significant and it unquestionably explains the unusually low protein and dry matter digestibility values contributed by this animal which were discussed before. Animal 7261S was assigned to the milk protein diet and was studied at 2 weeks of age.

Experiment 1083: Preliminary Observations  
on the Use of the Secretin Pancreatic Function  
Test on Anesthetized Baby Pigs

Purpose

In Experiments 1074, 1085, 1099, and 1114, protein and dry matter digestibility were consistently reduced by the exclusion of pancreatic secretion into the duodenum of 4-week-old pigs, and in Experiment 1114 of

2-week-old pigs. This result strongly suggests that the pancreas did in fact perform a digestive function at this early age, yet in Experiment 1074 and 1085, no pancreatic juice was issued from pancreatic fistulae of 4-week-old pigs. It seemed pertinent at this point to demonstrate that the pancreas of the 2-week or 4-week-old pig was capable of elaborating an exocrine secretion. It was decided that if the pancreas of the 2-week-old pig could be caused to secrete in acute experiments after stimulation with the secretin, it would demonstrate that the gland was capable of physiological excitation at this early age but it would not necessarily prove that the pig elaborated its own endogenous secretin. To the writer's knowledge the use of secretin for the study of pancreatic function in the species of animal used exclusively in this study has not been previously reported or described. Therefore it was the purpose of this experiment to establish the dosage of secretin that would be required to obtain a substantial pancreatic response in an anesthetized pig and to establish the course of events which follow intravenous injections of varying amounts of secretin. The evidence thus accumulated would be applied to the test of pancreatic function of young pigs.

#### Procedure

Two 10-week-old pigs were selected and fasted 24 hours before anesthetization. A pancreatic fistula was prepared as described in Experimental Methods by cannulating the pancreatic duct. A ligature was situated around the duodenum immediately below the pyloric sphincter to eliminate any possible gastric emptying into the small intestine.

A three channel continuous recording, E and M Physiograph<sup>a</sup> was used to record the blood pressure and pancreas secretion rate continuously throughout the investigation of one animal. Only the secretion rate was recorded of the second animal. The secretion rate was recorded by use of a drop counter and the blood pressure by cannulating the left carotid artery with the appropriate transducers for the physiograph. An injectable secretin solution was prepared with physiological saline. In a pilot trial which preceded this study a saline control was found to be negative. The secretin was injected, in successively larger doses, into the exposed jugular vein.

#### Results and discussion

The blood pressure recording of the first pig studied revealed that injections of 0.5, 1.0, 2.0, and 4.0 units of secretin per kilogram of body weight had no appreciable affect on the blood pressure. Thomas (129) stated that all early preparations of secretin caused a marked drop in blood pressure, but this was later found to be due to nonspecific tissue extracts.

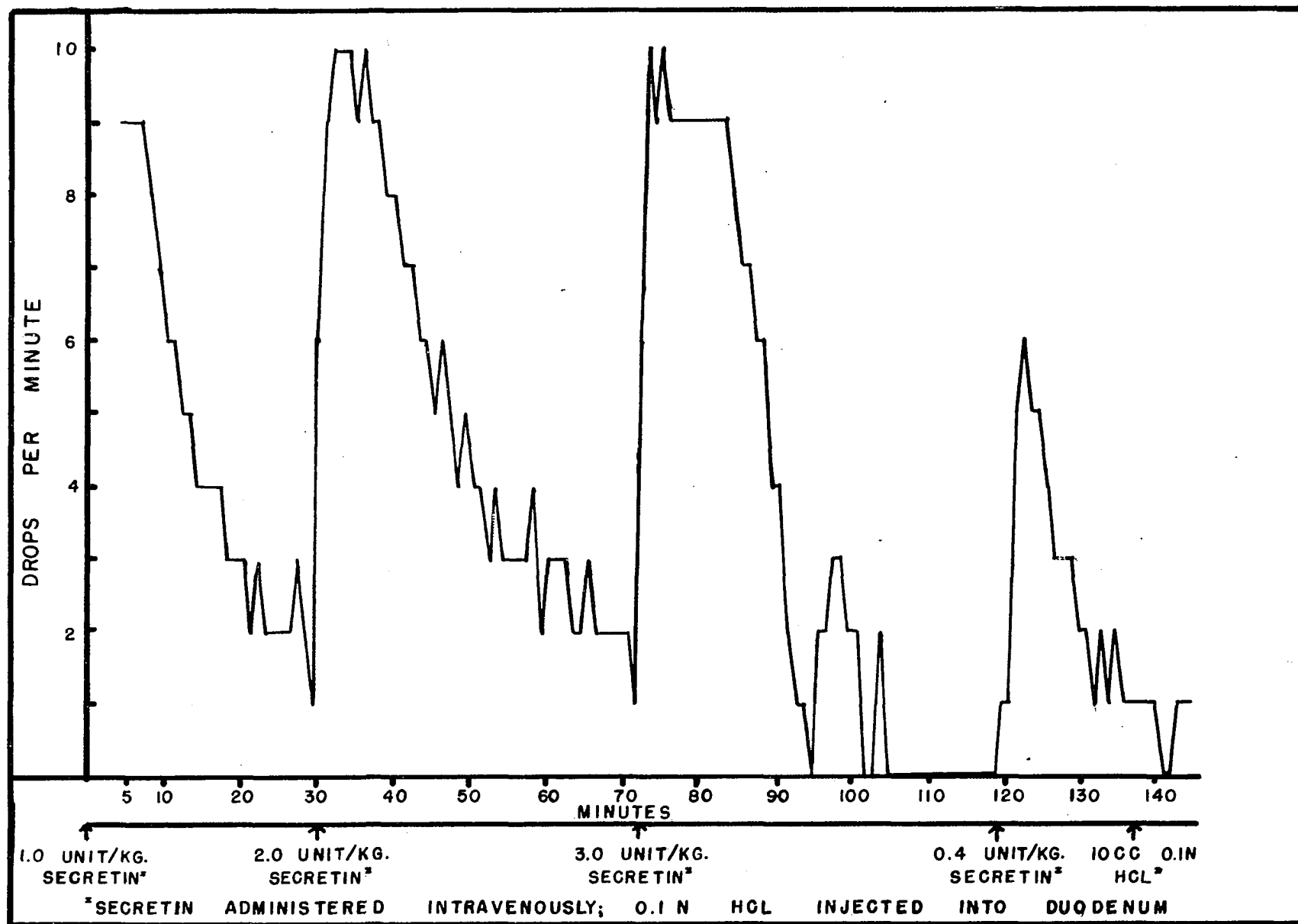
No pancreatic secretion was issued in response to injections of 0.5 and 1.0 unit secretin per kilogram body weight. However, 10 minutes after the injection of 2.0 units secretin per kilogram of body weight the cannula was gently resituated in the duct and the first secretion appeared at a considerable rate. Thus it was concluded that the lack of response

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<sup>a</sup>E and M Instrument Co., Inc., Houston 21, Texas

to the smaller doses was due to the failure of the pancreatic cannula. Immediately after the secretion appeared, a rate of 41 drops per minute was recorded which was followed by a linear decrease in the secretion rate to 10 drops per minute 32 minutes after the injection. This was followed immediately by an injection of 4.0 units secretin per kilogram body weight which caused the secretion rate to rise suddenly from 10 drops per minute to a high peak of 58 drops per minute only two minutes after the injection. The secretion rate decreased in a linear fashion so that 36 minutes after the secretin injection the rate had reached a plateau of eight drops per minute. From the results of this animal it was concluded that a 40 minute collection period would be adequate to collect all the secretion issued in response to relatively high doses of secretin (one unit of secretin per kilogram body weight is commonly used for the clinical pancreatic function test). The second animal was prepared with a pancreatic fistula only and the secretion rate was recorded as before. Pancreatic juice was secreted in response to an injection of 1.0 unit of secretin per kilogram body weight. However, it was found that the drop counting apparatus was accidentally pushed out of alignment and this explains the absence of a secretion rate plot during the first 4 minutes after the injection in Figure 13. The results summarized in Figure 13 indicate that the pancreatic secretion rate response to secretin reached a high peak in less than 5 minutes after the injection and returned to a near resting level 40 minutes after the injection. The total number of drops recorded after the injection of 1.0, 2.0, 3.0, and 0.4 units of secretin per kilogram body weight was 117, 209, 169, and 48 drops respectively. In the case of the

Figure 13. Graphic summary of the pancreatic secretion rate response following intravenous injections of 1.0, 2.0, 3.0, and 0.4 units secretin per kilogram body weight and intraduodenal infusion of 0.1 normal hydrochloric acid. Experiment 1083



two highest doses of secretin, 35 drops were secreted during the first 4 minutes after the hormone was injected and this would appear to be a reasonable correction for the total secretion issued in response to 1.0 unit of secretin since the value of 117 drops did not include the first 4 minutes; thus the total response would be 152 drops. The total secretion response to a single injection of 1.0, 2.0, or 3.0 units of secretin per kilogram body weight indicates that a near maximum response was obtained with 1.0 unit. Approximately 90 minutes after the first injection of secretin, respiration became irregular and the rate slowed; the pancreatic secretion rate dropped rapidly coincident with the respiratory complication. Injection of 0.4 units of secretin per kilogram body weight elicited an unquestionable pancreatic response but the total response was decidedly smaller than that elicited by the larger doses. No attempt was made to establish the possibility of or degree of exhaustion of the gland after each subsequent stimulation. After the gland returned to the resting state after the last secretin injection 10 milliliters of 0.1 normal hydrochloric acid was infused into the lumen of the duodenum. No pancreatic juice was secreted in response to the acid.

From these results it was concluded that the pancreas of the anesthetized pig could be excited to a secretory state by a single injection of secretin at a dosage of 0.4 units per kilogram body weight. Increasing the dosage to 2.0 units per kilogram body weight produced a maximal response, greater than that with 3.0 units. The infusion of 10 milliliters of 0.1 normal hydrochloric acid into the duodenum did not demonstrate a stimulatory effect on the pancreas. In each case the

pancreas was successfully stimulated by a single injection of secretin and the associated response and the subsequent return to the pre-injection state were expressed within 40 minutes after the injection.

Experiment 1084: Pancreozymin-Secretin  
Pancreatic Function Test of Baby Pigs  
at 3 and 6 Weeks of Age

Purpose

The purpose of this experiment was to determine if the pancreas of the 3- and 6-week-old baby pig could be excited to secrete pancreatic juice with digestive activities after an intravenous injection of pancreozymin and secretin and to investigate a possible diet and age influence on the pancreatic response to these hormones.

Procedure

Three unrelated groups of four littermate pigs were weaned from their dams at 7 days of age and placed on their respective experimental diet. A 2x2 factorial design was used consisting of the two experimental diets and two ages of animals. The basic experimental design was repeated three times. The pancreatic juice was collected for 40 minutes by the method described in Experimental Methods for the pancreozymin-secretin pancreatic function test. Pancreatic ducts were not cannulated directly due to the small size of the ducts of the 3-week-old pigs.

Results and discussion

The design and results of this experiment can be seen in Table 19. The test was successful with 5 of the 3-week-old pigs but pancreatic juice

Table 19. Summary of Pancreozymin-Secretin pancreatic function test on 3 and 6 week old pigs. Experiment 1084

Diet		Soybean protein		Milk protein	
Age (weeks)	Replication	3	6	3	6
Animal number	1	7351	7355	7354	7350
	2	7361	7360	7362	7363
	3	7377	7371	7372	7379
Animal weight at time of test, kilograms	1	4.73	11.72	6.00	15.14
	2	3.36	b	4.75	10.00
	3	2.55	b	3.05	b
Total volume secreted, milliliters	1	4.10	7.20	6.35	b
	2	4.00	b	a	4.70
	3	3.80	b	3.70	b
Total amylase secreted, gram maltose equivalent	1	3.72	4.53	14.48	b
	2	2.58	b	a	2.02
	3	0.66	b	2.73	b
Total protease secreted, milligrams trypsin equivalent	1	28.66	25.42	63.50	b
	2	33.16	b	a	12.74
	3	11.40	b	17.69	b
Total volume per kilogram body weight, milliliters	1	.87	.61	1.06	b
	2	1.19	b	a	0.47
	3	1.49	b	1.21	b
Total amylase per kilogram body weight, gram maltose equivalent	1	.79	.39	2.41	b
	2	.77	b	a	0.20
	3	.26	b	0.90	b
Total protease per kilogram body weight, milligram trypsin equivalent	1	6.06	2.17	10.58	b
	2	9.87	b	a	1.27
	3	4.47	b	5.80	b

<sup>a</sup>Pancreatic cannula filled with secretion but no pancreatic juice was collected in the collection vessel.

<sup>b</sup>No pancreatic juice secreted.

was obtained from only 2 of the 6-week-old pigs. In no case was there noticeable secretion by the gland before the hormones were injected. In those animals in which the pancreas was stimulated to secrete by the hormones, the volume response was not apparent after the administration of pancreozymin which was injected first but appeared abruptly only seconds after the secretin was injected. In each case the secretion stopped in less than 40 minutes. It is apparent from Table 19 that only two of the 6-week-old animals yielded a positive pancreozymin-secretin response. In the case of the 6-week-old group of animals it was felt that the lack of secretion was due to sloughing of the fragile and fecund mucosa of the intestine under negative pressures which occluded the cannula. Varying the pressure in the collection vessel from slight positive pressure, for an instant to clear any possible cannula blocks, to atmospheric pressure and then to negative pressures up to 1 centimeter of mercury did not result in a flow of secretion from the pancreatic fistula.

The results of the five 3-week-old pigs clearly indicate that the pancreas was physiologically capable of eliciting a response to pancreozymin and secretin, and that the magnitude of the response was similar for the pigs on each of the two experimental diets. The five 3-week-old pigs secreted an average of 1.16 milliliters of pancreatic juice per kilogram body weight after injection of 2.0 units each of pancreozymin and secretin. The total 40 minute output of digestive enzymes was not markedly different for the animals on the two diets.

## DISCUSSION

It is the purpose of this section to discuss those results which are generally supported by research described in the previous section of this dissertation. Emphasis is placed on those experimental results which relate directly to the experimental objectives defined in a previous section.

## Chronic Pancreatic Fistula Pig

Chronic pancreatic fistulae were prepared on ten baby pigs reported in this dissertation and on several other animals in the preliminary phases of the research program which were not discussed in this dissertation because they were not involved in a specific experimental design with specific objectives other than to develop the necessary methods and techniques for subsequent experiments. The method used here was similar to the method used by Elman and McCaughan (33), and Magee and Hong (96). It was found that the baby pig had to be partially restrained throughout the experimental period where an external pancreatic fistula was involved. This was necessitated by the disposition of the baby pig, the requirement that the animal remain relatively quiescent during the collection of pancreatic juice and the requirement that the animal would not be subjected to unnecessary excitement by abrupt changes in the environment and personnel. A satisfactory portable partial restraining collection unit was designed for this purpose. The animal was restrained within the collection unit by use of a harness. Feces and urine were accurately separated with this equipment by using male pigs.

In no case was pancreatic juice secreted from the fistula of baby pigs at 4 weeks of age or younger. Pancreatic juice was secreted in every case where a pancreatic fistula was established in pigs more than 6 weeks of age. This is not taken as proof that there is no pancreatic juice secreted into the duodenum of pigs below 4 weeks of age because in Experiments 1074, 1085, 1099, and 1114, where pancreatic digestion was excluded by ligation of the pancreatic ducts or by cannulating the pancreatic duct of pigs between 2 and 8 weeks of age, it was found that protein and dry matter digestibility were consistently reduced, regardless of the age of the pig; in Experiment 1084 it was demonstrated that the pancreas of the 3-week-old pig could be consistently stimulated to secrete after pancreozymin and secretin stimulation and the secretion elicited had appreciable enzyme activity. During the operative preparation of the fistula, of a few of the pigs below 4 weeks of age, a very slight amount of juice accumulated in the cannula. It is conceivable that the secretion of these young animals was prevented by a fibrin clot as a result of a tissue reaction in response to the cannulation procedure, whereas the formation of such a clot is prevented by a more profuse secretion in the case of the older animals.

The pancreatic fistula appeared to be completely successful in those cases where pancreatic juice was issued from the cannula shortly after recovery from the anesthetic. Attempts to return the pancreatic juice to the animal continuously by way of a duodenal fistula or intermittently by adding it to the drinking water and feed were not successful.

The writer feels obligated to state here that the problems encountered in the study of the external secretion of the pancreas are many and difficult. Pavlov (112, p. 59) found it appropriate to quote Heidenhain to best express his own experiences with the use of the pancreatic fistula in mature dogs. The writer of this dissertation finds this same quotation especially appropriate to express his experiences with pancreatic fistulae of young pigs: "Indeed, every observer who has been occupied for any length of time investigating the functions of the pancreas will leave this field with a feeling of dissatisfaction in consequence of the extremely large number of fruitless experiments he is obligated to subtract from the total number of his investigations; for not even the greatest care, nor the ripest experience in the making of pancreatic fistulae, will overcome the incomprehensible sensitiveness of the organ, which only too often annuls its function for a length of time after the operation, a function which it does not resume even under the influence of the most favourable secretory conditions. A degree of uncertainty, therefore, always clings to the results of such observations, which is not set aside even by frequent repetition of the experiments".

#### Pancreatic Secretion Rate

In Experiment 1026 the secretion rate was recorded continuously for periods up to 18 hours. It was demonstrated that the secretion rate was extremely variable (see Figures 5, 6, and 10) and difficult to explain. A coefficient of variation was calculated to be as high as 190 where the secretion rate was expressed as drops per ten minutes; see Table 9.

There seemed to be an association between the volume of juice secreted and the quantity of the diet consumed and also as association between the secretion rate and the presence of the investigator in the room where the experimental animals were maintained. These results suggest that the pancreas of the baby pig exhibits a cephalic phase and intestinal phase of secretion as described by Thomas (129). The data accumulated in these experiments seem to justify the statement that the rate of pancreatic secretion of baby pigs is subject to considerable variation probably because of the integration of psychic, nervous, and digestive influences on the gland. In these experiments the objective was to evaluate the spontaneous exocrine secretion of the gland. Therefore, although some attempts were made to reduce this variation by feeding the animals a test meal in a rhythmic schedule it must be realized that the more the experimental conditions are adjusted to reduce the variation, the less the secretion obtained will resemble the spontaneous secretion. Magee and Hong (96), Pavlov (112), Alphin and Lin (2) have reported that the pancreatic secretion rate varies greatly. Pavlov (112) has proven the existence of a psychic influence on the secretion of the pancreas of dogs. Harper and Vass (53) and Pavlov (112) and others have demonstrated that the pancreas is subject to complex nervous influences.

#### Composition of Pancreatic Juice

In Experiments 1026 and 1085, pancreatic juice was collected at frequent intervals during periods of 4 to 8 hours. It was found that the protease, amylase and lipase activities of the juice varied considerably.

It was demonstrated that the concentration of the enzymes vary independently of each other with no apparent association between them. This result is not in agreement with Babkin's "parallelism" theory. Due to the intended nutrient balance of the diets used in these experiments, the results cannot be taken to contradict or coincide with Pavlov's "purposive adaptation" theory. Guth et al. (45,46) could not accept either theory from their results with dogs. In Experiment 1085 it was observed that a pig fed a soybean protein diet test meal secreted about five times more protease, amylase, and lipase during a  $4\frac{1}{2}$  hour collection period and two times more volume of juice than a pig fed a milk protein diet test meal at precisely the same time and under exactly the same conditions. These results are not taken in full trust because it was found that the histological alteration of the pancreas tissues of the pig fed the milk protein diet was similar but more extensive than that of the pig fed the soybean protein diet. Similar diets were not compared by other investigators using pancreatic fistula and therefore these results stand alone. However, these results do contribute to the results obtained by Chernick et al. (16) and Lepkovsky et al. (81) who have demonstrated that the proteolytic activity of duodenal contents and pancreatic tissue of chicks fed a raw soybean meal diet is considerably greater than of chicks fed a similar diet with autoclaved soybean meal. Lyman and Lepkovsky (89) demonstrated the same effects of raw soybean meal on the enzyme activity in the intestine of the rat and found that the same effects could be produced by the inclusion of crystalline trypsin inhibitor in an autoclaved diet. They produced evidence that the excessive

proteolytic activity of duodenal contents originated in the pancreas. Lyman (88) concluded that the trypsin inhibitor, due to its stimulatory effect on the pancreas, increased the proteolytic activity in the intestine instead of decreasing it according to the earlier concept.

Although the diets used in Experiment 1085 were not similar to the diets used in the investigations discussed above (16, 81, 88, 89) the results similarly indicate a significant interaction between the diet and the physiology of the pancreas gland. If this relationship can be positively established in future investigations it would indeed be a significant and important contribution to the knowledge of nutrition and digestive physiology. It is unfortunate that no pancreatic juice was obtained in these investigations from pigs under 4 weeks of age to compare the pancreatic juice of pigs at various ages.

In summarizing the results of the preliminary studies associated with this research and including some of the results of Experiment 1026 reported in this dissertation, the writer, Pekas et al. (113) reported the range of values established for the concentrations of the various components of pancreatic juice of baby pigs near 5 weeks of age as follows: pH, 7.79-8.20; protease, 0.013-0.60 milligrams pancreatin (3 X U.S.P.) per milliliter; amylase, 54-772 milligrams maltose equivalents per milliliter; lipase, 0.033-0.353 milliequivalents potassium hydroxide per milliliter. Between 0.00 and 0.019 milliequivalents of acid were required to titrate 1.0 milliliter of secretion to the phenolphthalein end point. These values were not necessarily determined on the same samples. The secretion rate was reported to be between 0.0 and

11.0 milliliters per hour.

These values can now be supplemented to include the results of Experiment 1085 involving two 8-week-old pigs as follows: protease, 0.08-2.00 milligrams trypsin per milliliter; amylase, 711-3600 milligrams maltose equivalents per milliliter; lipase, 0.2 to 1.8 milliequivalents potassium hydroxide per milliliter; secretion rate, 2.3 to 10.5 milliliters per 30 minutes. The pH and buffering capacity of the secretion were not determined.

#### Nutrient Digestibility in the Absence of Pancreatic Juice

In Experiments 1099 and 1114 pancreatic digestion was intentionally excluded by ligation of the pancreatic duct. In Experiments 1074 and 1085 pancreatic digestion was excluded by cannulation of the pancreatic ducts. Seventy animals between 2 and 8 weeks of age were involved in these four experiments. Half of the animals were sham operated controls, six were prepared with pancreatic fistula (only two secreted pancreatic juice via fistula) and the remainder of the animals had ligated pancreatic ducts. In every case, the exclusion of pancreatic digestion reduced protein digestibility to a considerable degree and reduced dry matter digestibility to a lesser degree when compared to the respective sham operated control animals. In Experiments 1099 and 1114, the experimental designs and the number of animals used were adequate for statistical treatment of the data.

In summary it was found that the digestibility of protein and dry matter was significantly higher for the milk protein diet than the

soybean protein diet, was significantly reduced by ligation of the pancreatic duct and was increased significantly with advancing age. In addition, in Experiment 1114 a significant interaction between the dietary protein source and the pancreatic treatment was observed in the analysis of variance of the protein digestibility data; the data indicate that this was because the protein digestibility was reduced to a greater extent for the soybean protein diet than the milk protein diet by exclusion of pancreatic digestion. These results yield strong evidence that the pancreas plays a more prominent role in the digestion of the soybean protein than the milk protein diet. In Experiment 1085 it appeared that the degree of reduction of protein digestibility, due to the exclusion of pancreatic juice, increased for the soybean protein diet and decreased for the milk protein diet as the animals grew older. In Experiments 1099 and 1114 in which greater numbers of animals were involved this trend was reconfirmed. Karvinen et al. (69,70) found that exclusion of pancreatic digestion in the rat by ligation of the pancreatic duct significantly reduced the utilization of various fats as compared to sham operated control rats. Pavlov (112, p. 8) reported that ligation of the pancreatic ducts of dogs caused no detrimental effects; on the contrary Popper and Sorter (118) found that ligation of the pancreatic ducts of dogs resulted in death within 12 months.

#### Histopathological Alteration of Pancreatic Tissue After Ligation or Cannulation of the Pancreatic Duct

In Experiment 1085 it was found that the pancreas tissue, of two 7-week-old pigs with pancreatic fistulae, had atrophied and revealed

fibrosis which was most intense in the local regions of the cannula. The islets of Langerhans were difficult to distinguish in the pancreatic tissue of one animal and were not seen in the tissue of the other. The tissues of the sham operated control animals appeared normal under microscopic examination. In Experiments 1099 and 1114, 16 sham operated control pigs and 16 pigs with ligated pancreatic ducts were sacrificed for examination of the pancreatic tissue. With only one exception, the tissues of the sham operated control animals appeared normal whereas the tissues of the pigs with ligated pancreatic ducts revealed extensive atrophy and fibrosis and dilation of the pancreatic ducts. The islets of Langerhans were clearly distinguishable in the pancreatic tissues of both the pancreatic duct ligated pigs and the sham operated control pigs at 8 weeks of age; they were less distinct at 4 weeks of age; and they were indistinguishable at 2 weeks of age. The islets of Langerhans were found to be undergoing hydropic degeneration in the tissues of the 8-week-old pigs with ligated pancreatic ducts. The pancreatic ducts of the tissues obtained from pigs with ligated pancreatic ducts were generally found to be dilated. The pancreas tissues of sham operated control pigs and of the pancreatic duct ligated pigs were generally found to be infiltrated with lymphocytic cells which was thought to be due to stasis of the lymphatic flow caused by intestinal adhesions. Acini cells in the pancreatic tissue of 2-week-old pigs with ligated pancreatic ducts appeared to be less prominent and less numerous than normal. These histological alterations are in very good agreement with the results reported by Popper and Sorter (118), and Gibbs and Ivy (39), after the ligation of pancreatic

ducts of dogs.

Gross observations of the carcasses and viscera of these baby pigs did not reveal any differences between sham operated control pigs and the pancreatic duct ligated pigs. The wounds were found to be healing nicely in all cases examined. No active peritonitis was apparent, however, peritoneal adhesions between the intestines in the region of the laparotomy were common.

#### Pancreozymin-Secretin Pancreatic Function Test

In Experiment 1083 it was established that 0.4 units of secretin per kilogram body weight of a 10-week-old pig was sufficient to elicit a secretion of pancreatic juice and that increasing the dosage to 2.0 units per kilogram caused a greater volume of secretion response than 3.0 units per kilogram and it was further established that the secretion response and recovery to a single injection of the hormone occurred within a 40 minute period. In Experiment 1084, 2.0 units of pancreozymin and 2.0 units of secretin were injected intravenously in this respective sequence per kilogram of body weight to anesthetized pigs with pancreatic fistula. It was found that the volume, protease, and lipase output of the gland were not markedly different for three pigs fed the soybean protein diet and three pigs fed the milk protein diet. These pigs were 3 weeks old and the hormone response was very dramatic, illustrating that the pancreas of pigs at this young age are capable of elaborating pancreatic juice with digestive activity. A similar test was repeated on pigs 6 weeks of age, but due to technical difficulties secretion was obtained

from only two animals. The pancreozymin-secretin test is used for clinical diagnosis of pancreatic disorders. According to Sun and Shay (128) the pancreozymin-secretin test is a more reliable diagnostic test than the secretin test alone.

## SUMMARY

Eight experiments involving 134 baby pigs were conducted for the purpose of evaluating the quantitative output of digestive enzymes of the pancreas of baby pigs at various intervals between 2 and 8 weeks of age, the influence of a soybean protein diet and a milk protein diet on the pancreas secretion and the relative role of the pancreas in the digestion of these two diets. Pancreatic secretion was collected via a pancreatic fistula. Protein and dry matter digestibility were determined with baby pigs prepared with pancreatic fistula or with ligated pancreatic ducts and sham operated control animals. Special equipment and methods were designed and are described in this dissertation for the preparation and maintenance of pancreatic fistula pigs. Under the conditions of the experimental investigations reported herein the results obtained justify the following conclusions and statements; (1) milk protein produced faster and more efficient gains than soybean protein in the diets of baby pigs, (2) the rate of secretion and the composition of pancreatic juice are highly variable, (3) the rate of secretion frequently increases at a time coincident with or shortly before feeding (apparent cephalic phase of secretion), (4) a secondary and more sustained response in the rate of secretion was frequently observed after consumption of feed (apparent intestinal phase of secretion), (5) enzyme activities of pancreatic juice vary independently, which is not in agreement with the "parallelism" theory, (6) protein and dry matter digestibility were significantly reduced by the exclusion of pancreatic secretion into the duodenum,

(7) the reduction in digestibility was of a greater magnitude for pigs fed a soybean protein diet than pigs fed a milk protein diet, (8) the relative role of the pancreas in the overall digestive process does not appear to change between 2 and 8 weeks of age, (9) pancreatic juice was not obtained from pancreatic fistulae of pigs under 4 weeks of age but evidence was obtained which indicates that this was an experimental contingency, (10) the pancreas of the 3-week-old pig can be stimulated to secrete an enzyme rich juice with pancreozymin and secretin administration, (11) limited evidence suggests that a soybean protein diet effected a greater output of pancreatic juice and enzymes than a milk protein diet.

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## APPENDIX

Assay of Amylase by Means of Iodimetric Method  
for Measuring Reducing Sugars

Into a 50 ml. volumetric flask measure 25 ml. of 4% starch solution. Add 5 ml. of buffer (phosphate buffer, pH 6.9, consisting of 900 mls. 1 M  $\text{Na}_2\text{HPO}_4$ , 350 ml. 1 M  $\text{NaH}_2\text{PO}_4$ , 417 ml. 1 M NaCl and 417 ml. water), and approximately 18 ml. of water. Place the flask in a  $40^\circ\text{C}$ .  $0.05^\circ$  thermostatically controlled water bath and allow the starch to come to temperature. The enzyme solution is then added. Make up to volume with water. Mix well. Because of the instability of the amylases in solution, the enzyme solution should be prepared immediately before use. No attempt is made to bring the enzyme solution to  $40^\circ\text{C}$ . because of the danger of inactivation. The exact time at which the enzyme is added to the starch solution is noted. After exactly 30 minutes at  $40^\circ\text{C}$ ., 5 ml. of the starch digest is added to a 10 ml. volumetric flask containing 1 ml. of 10.6%  $\text{Na}_2\text{CO}_3$  solution. Water is added to make up to volume and mixed well. The  $\text{Na}_2\text{CO}_3$  will stop the action of the enzyme.

The reducing value of the digest is then determined as follows. Measure 2 ml. aliquots of the  $\text{Na}_2\text{CO}_3$  enzyme digest into 50 ml. glass stoppered flasks. Measurements are made in duplicate. Add 3 ml. of 0.02 N iodine solution and rinse the sides of the flask with 3 ml. of distilled water. Allow the iodine mixture to stand 30 minutes in a water bath at  $20^\circ\text{C}$ .  $0.5^\circ\text{C}$ . Add 1.0 ml. of 0.5 N  $\text{H}_2\text{SO}_4$  and titrate with 0.005 N  $\text{Na}_2\text{S}_2\text{O}_3$  solution, adding three drops starch solution indicator as the solution becomes pale yellow. Blank on the enzyme

solution is carried out using 2 ml. of the original enzyme solution plus 2 ml. of 1.06%  $\text{Na}_2\text{CO}_3$  plus 1 ml. of water plus 3 ml. of 0.02 N iodine solution. The iodine blank is determined by measuring 3 ml. of 0.02 N iodine solution, 2 ml. 1.06%  $\text{Na}_2\text{CO}_3$  and 3 ml. of water.

A blank on the starch solution is also necessary. This is determined by measuring 0.05 ml. 4% soluble starch solution (equivalent to 1 ml. digest) into a glass stoppered 50 ml. flask. Add 2.0 ml. 1.06%  $\text{Na}_2\text{CO}_3$ , 3 ml. 0.02 N iodine, and 2.5 ml.  $\text{H}_2\text{O}$  for rinsing flask. All blanks are allowed to stand in the 20° C. water bath for 30 minutes and titrated with 0.005 N  $\text{Na}_2\text{S}_2\text{O}_3$  in the same way as described for the digest.

### Calculations

The reducing value of the digest is calculated as follows:

- (1) Calculate first the enzyme blank for the sample titrated by subtracting the titration of the enzyme blank from the titration obtained for the iodine blank. This value divided by the appropriate dilution factor gives the enzyme blank for the sample of digest titrated.
- (2) The starch blank is calculated by subtracting the titration for the starch solution from the iodine blank.
- (3) The reducing value of the sample titrated equals (iodine blank minus enzyme blank minus starch blank minus sodium thiosulfate titration of the digest) multiplied by 0.855. 1 ml. of 0.005 N  $\text{Na}_2\text{S}_2\text{O}_3$  is equivalent to 0.855 mg. maltose. The sample titrated is equivalent to 1 ml. of the original digest. The reducing value of the sample titrated multiplied by

50 gives the actual mg. of reducing sugar calculated as maltose obtained from 1 g. of starch.