pair-feeding was used and weight gain before necrosis developed was alike in animals fed the choline-deficient diet alone and those given orotic acid in addition. However, since impaired growth decreases the frequency of renal necrosis (7), the lesser weight gain of animals given adenine sulfate could have obscured an ability to significantly nullify the protective action of orotic acid.

The mechanism by which orotic acid may lower the requirement for choline is obscure. Since phospholipid abnormalities may be of pathogenetic importance in both the fatty infiltration of the liver (10) and the renal necrosis (11) of choline deficiency, perhaps orotic acid influences phospholipid metabolism. Radioactivity can be recovered in hepatic cytidine nucleotides after administration of orotate-6-^{14}C to rats (12); it is conceivable, therefore, that orotic acid stimulates the cytidine-diphosphate-choline pathway of lecithin biosynthesis (13). The fact that hepatic levels of cytidine-diphosphate-choline are not depressed in choline deficiency (14) does not rule out this possibility.

Further study is needed to clarify the metabolic relationships between choline and orotic acid.

Summary. Addition of 1% orotic acid to a choline-deficient diet lowered the incidence of hemorrhagic renal necrosis in young rats from 86% to 41%, and within 24 hr prevented the accumulation of hepatic triglycerides by almost 50%. Simultaneous supplementation of the diet with 0.25% adenine sulfate did not influence these protective effects of orotic acid in choline deficiency.

It is suggested that orotic acid may lower the requirement of the body for choline through a metabolic interaction.

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Exocrine Function of the Chick Pancreas as Affected by Dietary Soybean Meal and Carbohydrate* (33448)

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Chicks develop an enlarged pancreas when fed unheated soybean meal. Autoclaving the meal destroys or inactivates the heat-labile component(s) which cause this effect on the pancreas (5). Recent histological studies in our laboratory (24) have confirmed that this enlargement is due to hyperplasia of the pan-

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creatic acinar cells, but the cause of this hyperplasia is not known (2). Homogenates of the enlarged pancreas have lower amounts of amylase, but higher levels of trypsin and chymotrypsin (20). Grossman *et al.* (9), and more recently Desnuelle *et al.* (6), suggested that differences in levels of pancreatic enzymes could be due to different rates of synthesis in response to dietary composition and/or an altered rate of secretion of enzymes from the pancreas. Most of the published results on pancreatic changes were obtained with pancreatic homogenates, a technique that precludes distinction between the suggested alternatives.

The purpose of the present study was to determine the effect of changing the nature, or source, of the dietary protein and carbohydrate on the volume and composition of the exocrine pancreatic secretion of chicks.

Experimental Procedure. Chicks were raised from 1 day of age to 3 weeks on four different diets containing autoclaved or unheated soybean meal with either glucose or cornstarch as the carbohydrate source. Composition of the basal diet has been described (21). Twelve birds (3/ treatment) were used for the pancreatic function studies and 36 (9/ treatment) for the determinations of intestinal pH. Chicks were anesthetized with pentobarbital sodium (20 mg/kg, intravenously) and the main pancreatic duct (of the three or four pancreatic ducts present in the chick) was cannulated using polyethylene tubing (PE 50). After recovery from anesthesia, normal feed consumption was observed. Beginning 2 days after cannulation, collections were made for 4 consecutive days. Samples were collected every 3 hr during the 14 hr of light provided to the chicks. Little, or no, juice was secreted during the night when chicks do not eat and the heaviest secretion was during the first hours of light in the morning. After collection, daily samples of pancreatic juice were pooled according to treatment and stored at —15° until time of analysis. Since only one pancreatic duct was cannulated, none of the collected pancreatic juice was returned to the duodenum.

Amylase, trypsin, chymotrypsin, and lipase were assayed on the samples. The 3, 5-dinitrosalicylic acid method was used for amylase determination (4). A unit of activity of this enzyme is expressed in terms of milligrams of maltose liberated in 3 min at 25°. Trypsinogen was activated with purified enterokinase (123-R) (2 mg/0.5 ml of juice) at 28° for 2 hr. Chymotrypsinogen was activated with trypsin (2 × crystalline) 50% $MgSO_4$ (0.1 mg/0.3 ml of juice) at 5° for 2 hr. (Enterokinase and trypsin were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.) Trypsin and chymotrypsin were analyzed spectrophotometrically using *p*-toluenesulphonyl-L-arginine methyl ester (TAME) and *N*-benzoyl-L-tyrosine ethyl ester (BTEE) as substrates (11), respectively. (All substrates were purchased from Sigma Chemical Co., St. Louis, Mo.). A unit of trypsin or chymotrypsin activity causes an absorbancy increase of 0.001/min at 25°. Lipase was determined using the titrimetric method with olive oil as the substrate (17). One unit of activity of the enzyme is equal to 1 μmole of acid produced per minute at 25° under the specified conditions. Specific activity is expressed as units of enzyme activity per milligram of protein. Protein was determined by the micro-Kjeldahl method (3). Total enzymatic activity values were calculated by multiplying the specific activities by the total protein secreted during the collection period.

The pH determinations of the duodenal contents were made with a Beckman model G pH meter; chicks were sacrificed after being on feed about 9 hr. Immediately after cervical dislocation, the duodenal loop was excised and the contents removed by manually forcing them through the open ends of the intestine.

Results. Pancreatic secretion and pH of the duodenal contents of the chicks on the different diets are presented in Table I. Daily secretion of pancreatic juice was higher for chicks fed unheated soybean meal as a source of protein. The difference was significant when starch was the source of carbohydrate but not in the case of glucose. The pH of duodenal contents was signnificantly increased when unheated soybean meal was fed, regardless of the type of carbohydrate.

TABLE I. Effect of Diet on the pH of Duodenal Contents of Pancreatic Secretion Rates.

Soybean meal treatment	Carbohydrate	pH[d]	Pancreatic secretion (ml/day/kg of body wt.)[e]	Total pancreatic juice protein (g)[f]
Autoclaved	Glucose	6.4[a]	9.7[ab]	1.20
	Starch	6.8[ab]	7.6[a]	0.97
Unheated	Glucose	6.6[bc]	11.7[b]	1.36
	Starch	6.8[c]	17.0[c]	1.51

[d] Each number represents the average of 9 determinations; means with the same letter are not significantly different at the 5% probability level.

[e] Average of 4-day secretion from 3 chicks.

[f] Total protein secreted during experimental period; calculated from average protein content and flow rate of pancreatic juice collected for 4 consecutive days.

Starch increased pH slightly, but not significantly. The higher secretion resulted, as would be expected, in higher pH values of duodenal contents since pancreatic juice is the main buffering agent for the intestinal contents.

The specific activities (Table II) of amylase, lipase, and chymotrypsin were slightly lower when unheated soybean meal and glucose were the components of the diet. Trypsin response was much the same with respect to the change in carbohydrate but, unlike the other enzymes assayed, its specific activity was slightly higher when unheated soybean protein was fed. The difference was not statistically different at the 5% probability level.

The effect of the different carbohydrates on the total activity of the enzymes (Table III) was similar to that observed in Table II. The inclusion of starch in the diet containing unheated soybean meal caused a larger amount of secretion of the enzymes analyzed, except for chymotrypsin in chicks fed autoclaved protein. The total activity of trypsin was statistically higher whereas other differences were not significant at the 5% probability level. Heat treatment of the soybean meal resulted in a decreased release of all four enzymes analyzed when starch was the dietary carbohydrate. When glucose was included in the diet, autoclaving the soybean meal resulted in a slight increase in the total activities of amylase, lipase, and chymotrypsin; furthermore, as in the case of diets with starch, trypsin was markedly depressed by heat treatment of soybean protein. This marked depression in tryptic activity was significant at the 5% probability level. Lower chymotrypsin-to-trypsin ratios were obtained with diets containing unheated soybean meal as compared with those of the diets containing the autoclaved meal.

Discussion. The inclusion of unheated soybean meal in the diet of chicks resulted in an increased pancreatic secretion, but the specific activity of the enzymes analyzed, with the exception of trypsin, was generally decreased. These results agree with the results of Pekas (19), who reported that unheated

TABLE II. Effect of Diet on the Specific Enzymatic Activity of Pancreatic Juice of Chicks.

Soybean meal treatment	Carbohydrate	Specific activity[b]			
		Amylase	Lipase	Trypsin	Chymotrypsin
Autoclaved	Glucose	43.49[a]	74.09[a]	96.36[a]	837.56[a]
	Starch	56.81[a]	125.67[a]	139.85[a]	990.70[a]
Unheated	Glucose	36.42[a]	61.09[a]	146.55[a]	566.82[a]
	Starch	50.40[a]	101.02[a]	147.93[a]	867.47[a]

[b] Means with the same letter are not significantly different at the 5% probability level.

TABLE III. Effect of Diet on the Total Enzymatic Activity of Pancreatic Juice of Chicks.

Soybean meal treatment	Carbohydrate	Total activity ($\times 10^3$)[c]				Chymotrypsin/trypsin
		Amylase	Lipase	Trypsin	Chymotrypsin	
Autoclaved	Glucose	52.19[a]	88.91[a]	115.63[a]	1005.07[a]	8.69
	Starch	55.10[a]	121.90[a]	135.65[a]	960.98[a]	7.08
Unheated	Glucose	49.53[a]	83.08[a]	199.31[b]	770.88[a]	3.87
	Starch	75.85[a]	152.04[a]	222.63[b]	1305.54[a]	5.86

[c] Means with the same letter are not significantly different at the 5% probability level.

soybean meal in the diet of pigs caused the secretion of a greater volume of pancreatic juice of a lower protein concentration.

The possibility of side effects of the surgical treatment on pancreatic secretion or composition of the juice cannot be disregarded even though chicks that undergo this operation seem to behave normally. Stimulation of secretion due to decreased amounts of pancreatic juice reaching the duodenum or to movements of the cannula could cause some of these effects. Another possible source of error is the cannulation of only one pancreatic duct of the 3 or 4 present in the bird. However, it has been reported that the responses from all cannulated ducts are parallel in the chick (10).

The increased secretion of trypsin observed in the pancreatic juice of chicks fed unheated soybean meal could be associated with the trypsin inhibitor present in soybean meal (SBTI). Lyman and Lepkovsky (16) reported that after administration of either a crude preparation of crystalline SBTI to rats, an increased secretion of pancreatic enzymes was observed as indicated by the high protease activity throughout the intestinal tract. Further studies by Applegarth *et al.* (2) showed that the feeding of unheated soybean meal to chicks caused a higher depletion of zymogen granules than in the ones fed autoclaved meal. This greater loss of zymogen granules was associated with larger decreases of proteolytic activity in these pancreases.

The mode of action of the trypsin inhibitor has not yet been elucidated. Snook (23) found that inclusion of egg white trypsin inhibitor (EWTI) in the diet of rats appeared to increase enzyme synthesis as estimated from assays of the pancreas 1 hr after feeding. These same assays performed 2.5 hr after feeding showed a decrease in zymogen content. Simultaneous measurements of intestinal tryptic activity indicated that enzyme secretion was unaffected or slightly increased. A possible explanation is an increased enzyme synthesis and secretion caused by EWTI, but increased intestinal tryptic activity was not detected in the presence of trypsin inhibitor. Another possible explanation of this decreased enzymatic activity in the intestine could be the formation of trypsin-inhibitor complexes (20). It was shown that this enzyme-inhibitor complex is stable for at least 3 hr under conditions similar to that in the intestine (1). Furthermore, the decreased proteolytic activity in the intestine might cause the pancreas to produce and secrete larger amounts of enzymes to try to compensate for this loss in activity (1, 5). Subsequent studies have shown that the ability of chicks to overcome the intestinal inhibition of proteolytic activity caused by raw soybean diets is influenced by both the age of the chick and the length of time the chicks were fed this diet (18). An increased secretion of proteolytic enzymes by the pancreas was suggested to be the mechanism by which the chick was able to overcome this intestinal inhibition. Also in support of this hypothesis are the results of Lepkovsky *et al.* (14), who observed that the proteolytic activity of the activated juice of chicks was markedly reduced by fractions of unheated soybean meal which were rich in trypsin inhibitors.

On the other hand, Lyman *et al.* (15) found that both egg white and lima bean trypsin inhibitor (LBTI) induced pancreatic secretion similar to that produced by the SBTI. After observing that inactivation of

the inhibitors with trypsin prior to feeding did not change the pattern of this response, the above mentioned authors concluded that the physiological activity of these substances was closely associated with their trypsin inhibitor activity but that inactivation of endogenous trypsin in the small intestine seemed not be be necessary for the response. The effect of the acidic environment of the stomach on the trypsin inactivated inhibitor could be an important factor affecting the results obtained by Lyman *et al.* (15). It has been shown that the soybean inhibitor complex is stable at pH 8, but that it dissociates rapidly into free trypsin and inhibitor below pH 4 (13). Therefore, it is possible that the inhibitor exerted its action on the pancreas while it was still free in the mildly acidic environment of the upper-and mid-duodenum.

The ratio of chymotrypsin to trypsin was lower in the pancreatic juice of birds fed unheated soybean meal as compared with the ones fed the autoclaved meal. This was expected as a result of the higher trypsin secretion of the birds fed the unheated meal. These values are higher than the ones reported by Snook (23), who found ratios of about 2.5 for the rat pancreas. These higher figures could be the result of using BTEE as the substrate for the assay of chymotrypsin instead of ATEE (acetyl-L-tyrosine ethyl ester) used by Snook (23). It has been reported before that chymotrypsin activity is severalfold greater when assayed with BTEE than with ATEE as substrate (12). Gorrill *et al.* (7) recently reported chymotrypsin to trypsin ratios ranging from 0.15 to 0.17 in pancreatic juice of calves fed soybean and milk protein diets. These results are markedly different from ours. Gorrill *et al.* (7) also mentioned that preliminary studies with chick pancreas indicated ratios of 1–2. Previous work in our laboratory (22) has indicated that the chick pancreas contains about 3 times more chymotrypsin than trypsin. The substrates used for the assay of these enzymes were the same, but slight differences in procedure, namely lower concentration of methanol used in our laboratory to dissolve the BTEE could account for this difference in results. It has been demonstrated before that the rate of hydrolysis of BTEE by chymotrypsin is dependent on the level of methanol in the reaction mixture (8).

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