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Changes in Pancreatic Enzymes as a Function of Diet in the Chick.* (32544)

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The phenomena of enzyme induction and repression are very well established in microorganisms, yet for vertebrates and mammals these have not been thoroughly investigated. The ratio of pancreatic enzymes is variable among species(5,9,12,13). Determinations of enzymes in homogenates of pancreata from rats fed either starch-rich or casein-rich diet show that specific activities of amylase and chymotrypsin reflect the starch and casein content of the diet(15). In chicks, the pancreata enlarge when unheated soybean meal is consumed. These pancreata show depletion of zymogen granules to a greater extent than the corresponding pancreata from chicks fed autoclaved soybean meal diet. This histological evidence was confirmed by chemical analysis(1). We investigated the rate of synthesis of enzymes by the pancreas of chickens after an adaptation period of 4 weeks on either unheated or autoclaved soybean meal.

Experimental. Two groups of chicks were fed either unheated or autoclaved soybean meal for 4 weeks. The composition of the basal diet was essentially that used by Dal Borgo(4). After this adaptation period, the chicks were fasted; 3 birds of each group were sacrificed at 0, 6, 12, 16, and 22 hours after the start of fast. After 16 hours of fasting, each group was divided into 2 subgroups, one of which was fed the same diet as before the fast, and the other the alternate diet. Three chicks from each of the 4 subgroups were sacrificed after 2 and 6 hours on the diet. The pancreata were excised and weighed (after removal of extraneous tissue), then stored at −15°C.

Trypsin and chymotrypsin were assayed using p-toluene-sulphonyl-L-arginine-methyl ester (TAME) and N-Benzoyl-L-tyrosine ethyl ester (BTEE), respectively(7). Amylase activity was determined by the 3,5,-dinitrosalicylate method(3). Protein in the pancreatic homogenates was determined by measuring the absorbancy at 280 and 260 mμ to correct for nucleic acids(10).

A portion of the pancreas was homogenized in H_2O for determination of amylase activity, while another part of the same pancreas was homogenized in 0.25 M sucrose for analysis of trypsin and chymotrypsin. The homogenote was diluted 10-fold with a 0.05 M phosphate buffer at pH 7.8 for chymotrypsin determination. Trypsinogen was converted to trypsin by activation with 4.0 mg enterokinase per 100 mg of tissue for 2 hours at 28°C. Crystalline trypsin, 0.01 mg/mg of protein, was found adequate for activating chymotrypsinogen to chymotrypsin at 5°C in 2 hours. Purified enterokinase (1-123R) and

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trypsin (2X crystalline) 50% $MgSO_4$ were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. A Bausch and Lomb Spectronic 600 spectrophotometer was used for protein, chymotrypsin and trypsin determinations, whereas a Beckman Model B was used for amylase assays.

A BTEE unit of chymotrypsin activity is that causing an increase of absorbancy in one minute of 0.001 at 25°C. A unit of trypsin activity causes an absorbancy increase of 0.001 per minute at 25°C. A unit of amylase activity is defined as the milligrams of maltose formed in 3 minutes at 25°C. Specific activity is defined as enzyme units per milligram of protein.

Results and discussion. Growth depression and pancreatic enlargement were observed in all chicks fed unheated soybean meal. Furthermore, the pancreata were pale and white compared with the pink pancreata from chicks fed autoclaved meal. This is not in agreement with results reported by Lepkovsky (11). A higher incidence of intestinal hemorrhages was also noticed in chicks consuming unheated soybeal meal.

Enzyme activity in the fasted chicks. The rate of synthesis of amylase in chicks fed unheated soybean meal was lower in comparison with chicks fed autoclaved soybean meal, based on the specific activity (Fig. 1). The total activity of pancreatic amylase was higher when chicks had been fed the unheated soybean meal (Fig. 2). This may be due to the size of the pancreas, which is about twice as large as the normal pancreas. The latter is significant because observations in this laboratory (unpublished) indicate that protein per unit weight is not different in either the normal or enlarged pancreas. Chymotrypsin specific activity did show increased rate of zymogen synthesis in the pancreata from the chicks on unheated soybean meal (Fig. 3), whereas trypsin was about twice as high with unheated as with autoclaved soybean meal (Fig. 4). Both total trypsin and chymotrypsin activities were much higher when unheated soybean meal was fed and in this respect they followed much the same pattern as amylase (Fig. 2, 5, 6).

Enzyme activity in pancreata of chicks fed the same diets before and after 16 hours of fast. The specific activity of amylase and chymotrypsin did tend to decrease at a faster rate in pancreata of chicks fed autoclaved soybean meal as compared with pancreata from chicks fed unheated soybean meal. The converse was true for the specific activity of trypsin. In all cases where autoclaved soybean meal was fed, a tendency for recovery was shown, as evidenced by the increase in specific activity of amylase, trypsin, and chymotrypsin (Fig. 1, 3, 4). The recovery was not observed when unheated soybean meal was fed, but on the contrary, the figures show a continuous depletion. Total amylase, trypsin, and chymotrypsin activity followed much the same pattern as the specific activities (Fig. 2, 5, 6). These results indicate that over an extended period of time, the pancreata from chicks fed unheated soybean meal would be depleted of their zymogen to a larger extent, perhaps due to the failure to resynthesize more zymogen. Apparently, zymogen is synthesized when autoclaved soybean meal is fed.

Enzyme activity in pancreata of chicks fed the alternate diet after 16 hours of fast. Both the specific and total activity of amylase decreased rapidly and no recovery was noticeable when unheated soybean meal was fed to chicks which had been adapted previously to the autoclaved diet. This is in contrast with a slight decrease in activity, followed by recovery, due to synthesis of the zymogen when autoclaved soybean meal was fed to chicks which previously had been adapted to unheated soybean meal (Fig. 1, 2). A similar pattern was observed for the total chymotrypsin activity with either diet (Fig. 5) and the specific activity of chymotrypsin when unheated soybean meal was fed. Pancreata of chicks fed autoclaved soybean meal showed a lower depletion. No recovery was shown on the basis of specific activity (Fig. 3). Both the specific and total activities of trypsin decreased and did not return to normal when either autoclaved or unheated soybean meal was fed (Fig. 4, 6).

The continued depletion of zymogen from the pancreas when unheated soybean meal is fed as the dietary source of protein is in

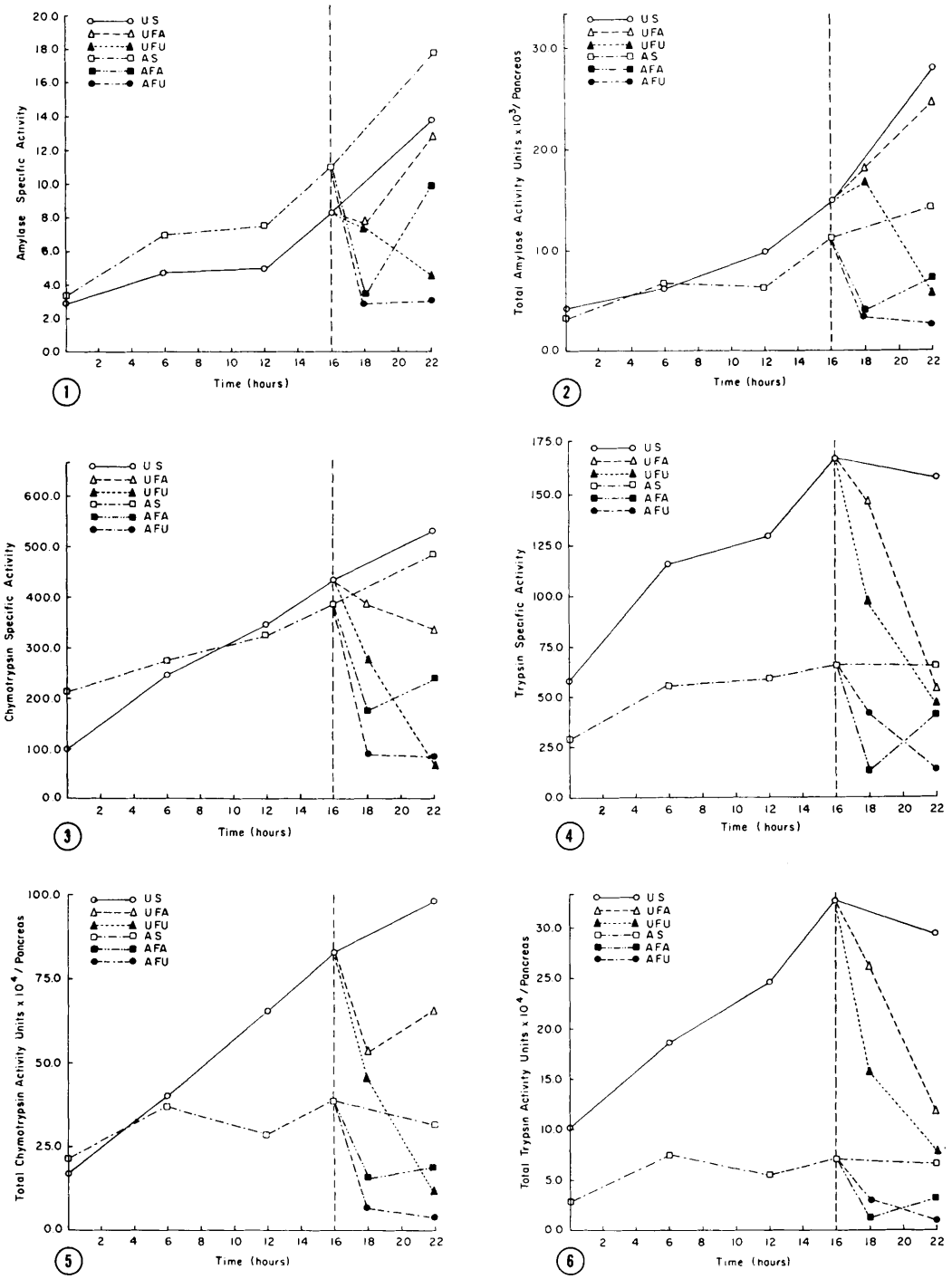

FIG. 1. Amylase specific activity in the pancreas of 4-week old chicks fed soybean meal with respect to time. Letters designate type of diet (U, unheated; F, fed; A, autoclaved; S, fasted). 1st letter is diet fed prior to fast; 2nd letter is state of chicks, fed or fasted; 3rd letter is diet fed after 16 hr fasting).

FIG. 2. Total amylase activity in the pancreas of 4-week old chicks fed soybean meal with

respect to time. Letters designate type of diet (U, unheated; F, fed; A, autoclaved; S, fasted). 1st letter is diet fed prior to fast; 2nd letter is state of chicks, fed or fasted; 3rd letter is diet fed after 16 hr fasting).

FIG. 3. Chymotrypsin specific activity in the pancreas of 4-week old chicks fed soybean meal with respect to time. Letters designate type of diet (U, unheated; F, fed; A, autoclaved; S, fasted). 1st letter is diet fed prior to fast; 2nd letter is state of chicks, fed or fasted; 3rd letter is diet fed after 16 hr fasting.

FIG. 4. Trypsin specific activity in the pancreas of 4-week old chicks fed soybean meal with respect to time. Letters designate type of diet (U, unheated; F, fed; A, autoclaved; S, fasted). 1st letter is diet fed prior to fast; 2nd letter is state of chicks, fed or fasted; 3rd letter is diet fed after 16 hr fasting.

FIG. 5. Total chymotrypsin activity in the pancreas of 4-week old chicks fed soybean meal with respect to time. Letters designate type of diet (U, unheated; F, fed; A, autoclaved; S, fasted). 1st letter is diet fed prior to fast; 2nd letter is state of chicks, fed or fasted; 3rd letter is diet fed after 16 hr fasting.

FIG. 6. Total trypsin activity in the pancreas of 4-week old chicks fed soybean meal with respect to time. Letters designate type of diet (U, unheated; F, fed; A, autoclaved; S, fasted). 1st letter is diet fed prior to fast; 2nd letter is state of chicks, fed or fasted; 3rd letter is diet fed after 16 hr fasting.

support of earlier studies with pancreatic homogenates(1) and with flow of pancreatic juice(8). Work in progress in our laboratory indicates that both the flow of pancreatic juice and the trypsin activity per unit volume are higher when unheated soybean meal is fed.

The higher specific activity of amylase in pancreata from chicks fed autoclaved soybean meal confirms the results reported by other investigators(4,11,14).

After observing that glucose induced the same pancreatic response as starch in rats, Ben Abeljlil and Desnuelle(2) suggested that the exocrine pancreas receives its information from the products formed during digestion rather than from the ingested products. This seems to be contrary to results obtained in the chick(4). Unheated soybean meal is less digestible than autoclaved soybean meal, and it may be assumed to be less hydrolyzed, due to its protein structure and bound inhibitors. This difference in the degree of hydrolysis of the protein may be one contributing factor to the changes in levels of the enzymes presented here. These results are supported by Howard and Yudkin(6), who postulated that the ratio of amino acids released during *in vivo* digestion of casein differs from the ratio obtained by complete hydrolysis of the protein. This postulate was made to explain their failure to find an identical response of enzymes after feeding casein or a tryptic hydrolysate of casein.

The difference in rate of pancreatic secretion observed after feeding either of the two diets in this study may be the result of variation in the peptides and the amino acids in the intestinal tract released by action of the enzymes on the dietary protein. In addition, the amino acid pool in the acinar cell also may regulate the synthesis of these enzymes. It was shown that inclusion of egg white trypsin inhibitors increases the rate of enzyme synthesis in the pancreas(16). Furthermore, it was suggested that the increased rate of digestive enzymes could be attributed to a higher relative rate of hydrolysis of dietary protein or to a regulatory mechanism on the pancreas by the dietary protein.

The presence of trypsin and chymotrypsin inhibitors, as well as other unidentified, deleterious factors in unheated soybean meal, causes incomplete digestion. Apparently, enzyme-inhibitor complexes are formed. This would result in an increased demand for protein synthesis and subsequent secretion of digestive enzymes by the acinar cells of the pancreas to counteract the effect of inhibitors. Pancreata from chicks which had been fed unheated soybean meal enlarge in size, apparently to meet this physiological demand for enzyme production.

Our data indicate that the pancreatic enzymes are synthesized according to dietary composition. In addition, synthesis of digestive enzymes is higher in pancreata of chicks adapted to unheated soybean meal. Enzyme activity was lower when unheated soybean meal was fed after the fasting period, possibly due to failure to increase synthesis to compensate for continuous depletion. This indi-

cates that enzyme synthesis is reduced in the enlarged pancreas when unheated soybean meal is fed. The rate of release of zymogen was higher whenever unheated soybean meal was fed, regardless of the diet fed during the adaptation period.

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Electrical Stimulation and Digitalis Drugs: Repetitive Response in Diastole.* (32545)

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Electrical shock, such as is employed in cardioversion, may induce serious cardiac arrhythmias in the digitalized patient(1). In the experimental animal, digitalization lowers the electrical threshold for ventricular tachycardia by a factor of 2000(2). The change in electrical threshold develops when about 85% of the toxic glycoside dose has been administered. Since in these studies the electrical discharge was administered transthoracically, it is uncertain whether the digitalis-induced sensitization resulted from the high energies employed. If digitalization similarly alters the response to small pacemaker stimuli delivered directly to the heart, utilization of this phenomenan may provide a method for estimating the degree of digitalization.

The present experiments demonstrated that small pacemaker stimuli, when administered to the digitalized animal, evoke repetitive ventricular responses. It was also noted that during digitalization the ventricular cycle becomes strikingly differentiated in its sensitivity to small test pulses.

Materials and methods. Fifteen mongrel dogs of both sexes, weighing 18 to 22 kg, were anesthetized by intravenous sodium pentobarbital in a dose of 30 mg/kg. Artificial respiration with room air was accomplished by a Harvard ventilatory pump through a cuffed endotracheal tube. A multistrand insulated steel electrode wire with a platinum tip of 0.5 mm in diameter was introduced through the jugular vein and floated into the

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