# Digestive Enzymes of the Baby Pig. Pancreatic and Salivary Amylase

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**The pancreatic tissues of pigs, from 1 to 42 days of age, were examined for amylase and maltase activities. Total amylase activity per pig increased in pigs from 1 to 35 days of age. The maltase activity per pig per gram of dry pancreatic tissue was so low that the role of pancreatic maltase in the baby pig is probably of little significance. The saliva of the baby pig, from 1 to 49 days of age, was comparatively low in amylase activity.**

THE ABILITY of the baby pig to utilize certain carbohydrates in feeding experiments conducted at this station indicated that the amylolytic enzyme system of the baby pig may not be fully developed.

Hudman and coworkers (*4*) have shown, in pigs weaned at 3-day intervals from 3 to 21 days of age and placed on a diet containing cornstarch as the only carbohydrate ingredient, that the 3-day-old weaned pig grew slower than pigs weaned at 6 days or older. Also, these workers have shown that an increased level of dried skim milk from 0 to 20 to 40% in the diet resulted in a significant linear increase in both gains and feed efficiency in baby pigs from 1 to 3 weeks old. Both the carbohydrate and protein fraction of dried skim milk contributed to the superior performance over semipurified soybean oil meal–cornstarch diets. This work indicated that cornstarch is not well utilized by the baby pig.

Recently, Kitts, Bailey, and Wood (*6*) have found that amylase activity of pancreatic extracts of baby pigs increases from negligible levels at birth to high levels after 21 days. These workers suggested that amylase production undergoes a significant increase at about the fourth week of life.

The extent to which pig salivary amylase is involved in food digestion has received little serious contemplation. In 1887, Ellenberger (*2*) showed, by the use of an esophageal fistula, that the sugar content of potatoes fed to pigs increased from 0.35 to 2.00% after chewing, due to the action of ptyalin (salivary amylase). Bergeim (*1*) maintained that salivary amylase activity may continue in the human stomach for about 15 minutes until gastric activity completely prevented further action. Ivy, Schmidt, and Beazell (*5*) substantiated this concept and further indicated that, in a mixed meal containing a moderate amount of substances which would buffer acidity, practically complete starch digestion by salivary amylase in the human stomach was possible.

Results of work with other species lead to the assumption that salivary enzyme concentrations in the pig may respond to dietary differences. Evans (*3*) demonstrated an augmentation of amylase in the saliva of humans fed carbohydrate diets. Squires (*13*) estimated the salivary amylase activity of three groups of subjects in Bechuanaland and concluded that salivary amylase concentration was conditioned by the amount of carbohydrate in the diet. Scatena (*10*) reported that breast-fed human infants had a higher ptyalin content than those artificially fed and that a mixed diet elicited an even higher concentration.

The purposes of these investigations were to determine the amylase and maltase activity of pancreatic tissue of baby pigs from birth to 42 days, and to measure the development of salivary amylase activity of baby pigs from birth to 49 days.

## Materials and Methods

**Pancreatic Amylase and Maltase.** The selection of pigs, removal of glands, and solvent drying of tissues have been described (*7*). The disk-plate method of enzyme assay (*7*) was adapted to the estimation of pancreatic amylase by making the following modifications: The substrate consisted of 0.09% sodium chloride, 0.2% soluble starch, and 1.0% agar in 0.066*M* phosphate buffer, pH 6.8. Following the incubation of the plates, the paper disks were removed; the plates were flooded with 0.025*N* iodine-potassium iodide solution; and excess iodine was poured off. The diameters of the zones of hydrolysis were read on a Fisher-Lilly antibiotic zone reader.

The data were also examined by the standard parallel-line assay analysis, with the same type of discrepancies in the results as described before (*7*).

Pancreatic amylase was also determined by measuring the increase in reducing sugars (milligrams of maltose) formed from starch. To 20 ml. of 0.1% soluble starch in 0.066*M* phosphate buffer, pH 6.8, 9.0 ml. of water and 1.0 ml. of enzyme solution were added—all equilibrated to 40° C. Two 2-ml. aliquots were tested by the Somogyi-Nelson (*8*, *12*) procedure at the time of addition of enzyme and 20 minutes thereafter. Using the 0-hour control as a blank, the absorbance of the 20-minute test was read in a Coleman Jr. Model spectrophotometer at 590 mμ. Results obtained were converted to maltose equivalents by comparison with a standard maltose curve.

Pancreatic maltase was estimated by measuring the increase in reducing substances (milligrams of dextrose) obtained after incubation of the enzyme solution for 1 hour at 40° C., with a substrate of 0.02% maltose in 0.066*M* phosphate buffer, pH 7.2.

**Salivary Amylase.** Three sows farrowing the same day were selected, with four sow and four boar pigs in each litter. During the first 7 days, six pigs were needed as replacements for weak or dead pigs. The boar pigs were castrated at 7 days of age. Sows and litters were kept in farrowing stalls for the first 8 days, then transferred to two unheated wooden buildings. Each lit-

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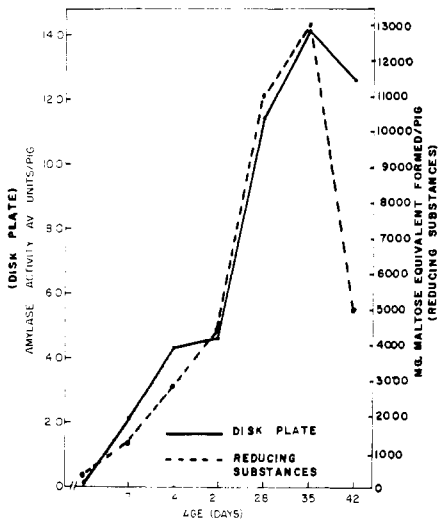


Figure 1. Amylase activity of dry pancreas tissue from baby pigs of various ages

ter was divided into two groups of four pigs each; the first group remained with the sow and the second group was weaned onto a casein-cornstarch diet. A heat lamp, self-feeder, and automatic water were provided for each pen of early weaned pigs. The composition of the diet fed to the early weaned pigs is given in Table I.

The sow was separated from her litter twice daily for feeding. Weekly doses of an iron-containing paste were given to all pigs suckling sows.

Saliva was collected for analysis when the baby pigs were 1, 7, 14, 21, 35, and 49 days old. To stimulate salivation, each pig was injected intraperitoneally with about 1 mg. per pound body weight of pilocarpine hydrochloride in saline. As soon as the flow of saliva started, usually about 5 minutes after administration of pilocarpine, each pig was held with its head down to facilitate collection of saliva into clean test tubes. Amylase activity of the saliva samples was determined by the same spectrophotometric procedure reported above for pancreatic amylase, except that results were recorded in maltose equivalents per milliliter of saliva.

**Table I. Composition and Analysis of Cornstarch-Casein Diet for Early Weaned Pigs**

| *Ingredients* | % |
|---|---|
| Cornstarch | 54.42 |
| Casein (crude) | 22.30 |
| Cane sugar (sucrose) | 10.00 |
| Beet pulp (dried) | 2.00 |
| Lard (stabilized) | 2.50 |
| Salt (iodized) | 0.50 |
| Trace mineral mixture (35-D-10)[a] | 1.63 |
| Dicalcium phosphate | 3.85 |
| Calcium carbonate | 0.80 |
| Vitamin and antibiotic premix[b] | 2.00 |
| CALCULATED ANALYSIS | |
| Protein | 20.01 |
| Fat | 2.51 |
| Fiber | 0.40 |
| Calcium | 1.24 |
| Phosphorus | 0.85 |

[a] Milligrams added per pound of ration: Fe, 164.6; Cu, 3.5; Co, 14.1; Zn, 14.7; Mn, 46.1; K, 1806.9; $I_2$, 0.2; Mg, 240.1. [b] Added per pound of ration: vitamin E, 10.0 mg.; ascorbic acid, 300.0 mg.; biotin, 20.0 $\gamma$ calcium pantothenate, 7.0 mg.; choline chloride, 450.0 mg.; folic acid, 9.0 $\gamma$; inositol, 250.0 mg.; menadione, 1.0 mg.; niacin, 29.8 mg.; *p*-aminobenzoic acid, 8.0 mg.; pyridoxine hydrochloride, 1.2 mg.; riboflavin, 5.0 mg.; thiamin hydrochloride, 5.0 mg.; vitamin A, 5000 I.U.; vitamin $D_2$, 1008 I.U.; vitamin $B_{12}$, 20.0 $\gamma$; chlortetracycline, 50.0 mg.

## *Results and Discussion*

**Pancreatic Amylase and Maltase.** The pig weights and wet and dry pancreas weights have been reported (*7*). The calculated amylase activities per gram of dry pancreatic tissue, determined by the disk-plate and spectrophotometric assays, are shown in Table II. A comparison of the two methods of analysis is graphed in Figure 1.

The average amylase activity per gram of dry pancreatic tissue was very low at 1 day of age, with a rapid, overall increase in amylase activity up to 28 days. However, there appeared to be a lowered rate of increase of amylase concentration from 14 to 21 days of age. After 28 days the amylase activity remained at about the same level.

Considering the average pancreatic amylase activity per pig, as shown in Figure 1, there is an increase from 1 to 35 days of age. A very slight increase is noted from 14 to 21 days. This was a period of a large increase in pancreas weight but with only a slight increase or decrease in amylase activity per gram. The reduced amylase activity per pig from 35 to 42 days of age may be explained partially on a pig-weight basis, as the weight per pig averaged about 1.6 pounds less for the 42-day-old pigs than for 35-day-old pigs. Also, only two pigs were used in making the 42-day tests, while the other points each represent the average of five or six pigs per group.

The results of this experiment are in relatively close agreement with the work reported by Kitts, Bailey, and Wood (*6*), in that these workers reported that the amylase activity per gram of dry pancreatic tissue increased with age from 1 to 35 days. However, the values reported herein (average of five or six pigs per age group) or in the above work (*6*) (average of one or two pigs per age group) should not be considered absolute values. Shay, Sun, and Gruenstein (*11*) have reported that large groups of rats are necessary to obtain reasonably reliable estimates of the enzyme content of this animal. A comparison of the two methods of analysis of pancreatic amylase (Figure 1) shows that considerable laboratory error is involved in assays of the type described herein when only a few animals are used per age group. Management, breed of pigs used, and other variables may affect the results obtained.

Pancreatic maltase activities, as represented by reducing substances are shown in Table III. The values recorded per pig and per gram of dry pancreatic tissue were so low that the role of pancreatic maltase in the baby pig is probably of little significance.

**Salivary Amylase.** As this work was considered preliminary, no statistical analysis of results was contemplated. Furthermore, although its exact mode of action is not fully understood, pilocarpine was used to incite salivary secretion.

The development of salivary amylase activity, expressed as milligrams of maltose equivalent formed from a starch substrate by 1 ml. of undiluted saliva, is shown in Figure 2. After an initial decline during the first 7 days, salivary amylase activity (although comparatively weak at all ages) increased in both groups of baby pigs. The peak of activity was reached at 14 days of age for the early weaned pigs and at 21 days for the suckled pigs. However, the activity of both groups of pigs had fallen to the same level by 35 days. The levels of salivary amylase remained fairly constant for the rest of the experimental period.

As the early weaned pigs reached a peak of salivary amylase activity earlier than the suckled pigs, a dietary adaptation of the salivary amylase concentration appears to be possible. However, temporary restriction of growth by early

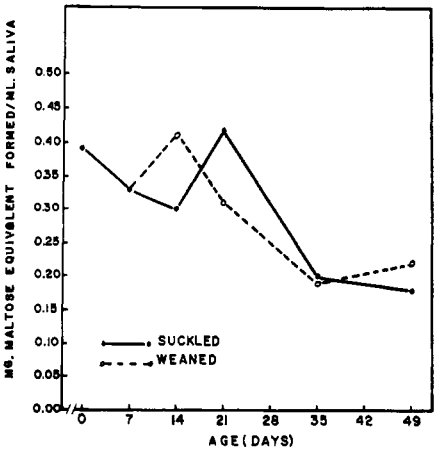


Figure 2. Amylase activity of saliva from baby pigs of various ages

**Table II. Amylase Activity of Pancreatic Tissue Extracts from Baby Pigs**

| Litter No. | Age, Days 1 | 7 | 14 | 21 | 28 | 35 | 42 |
|---|---|---|---|---|---|---|---|
| | Units of Activity/G. of Dry Tissue (disk plate) | | | | | | |
| 1 | 0.27 | 2.0 | 4.0 | 3.0 | 3.5 | 5.4 | .. |
| 2 | 0.28 | 2.2 | 4.2 | .. | .. | .. | .. |
| 3 | 0.16 | 2.8 | 4.0 | 3.0 | 3.7 | 5.1 | 4.1 |
| 4 | 0.18 | 2.3 | 4.5 | 3.4 | 6.4 | 4.8 | 5.8 |
| 5 | 0.25 | 3.3 | 2.0 | 2.3 | 5.1 | 3.4 | .. |
| 6 | 0.25 | 2.0 | 4.1 | 2.7 | 6.1 | 5.8 | .. |
| Av. | 0.23 | 2.4 | 3.8 | 2.9 | 5.0 | 4.9 | 5.0 |
| | Milligrams of Maltose Equivalent Formed/G. of Dry Tissue | | | | | | |
| 1 | 280 | 1000 | 2400 | 6000 | 6400 | 9500 | .. |
| 2 | 1880 | 1800 | 3600 | .. | .. | .. | .. |
| 3 | 440 | 2300 | 3840 | 1200 | 4400 | 2000 | 1800 |
| 4 | 540 | 1000 | 3000 | 2200 | 4500 | 4400 | 2200 |
| 5 | 400 | 1400 | 960 | 1300 | 3300 | 1200 | .. |
| 6 | 360 | 800 | 340 | 1800 | 3500 | 3000 | .. |
| Av. | 650 | 1380 | 2360 | 2500 | 4420 | 4020 | 2000 |

**Table III. Maltase Activity of Pancreatic Tissue Extracts from Baby Pigs**

| Litter No. | Age, Days 1 | 7 | 14 | 21 | 28 | 35 | 42 |
|---|---|---|---|---|---|---|---|
| | Milligrams of Dextrose Equivalent Formed/G. of Dry Tissue | | | | | | |
| 1 | 0.076 | 0.019 | 0.114 | 0.152 | 0.076 | 0.152 | ... |
| 2 | 0.114 | 0.152 | 0.057 | ... | ... | ... | ... |
| 3 | 0.076 | 0.076 | 0.038 | 0.057 | 0.152 | 0.152 | 0.171 |
| 4 | 0.019 | 0.076 | 0.057 | 0.038 | 0.152 | 0.095 | 0.076 |
| 5 | 0.152 | 0.133 | 0.114 | 0.095 | 0.038 | 0.057 | ... |
| 6 | 0.057 | 0.076 | 0.000 | 0.114 | 0.076 | 0.095 | ... |
| Av. | 0.082 | 0.089 | 0.063 | 0.091 | 0.099 | 0.110 | 0.124 |
| | Milligrams of Dextrose Equivalent Formed per Pig | | | | | | |
| 1 | 0.041 | 0.015 | 0.165 | 0.319 | 0.248 | 0.608 | ... |
| 2 | 0.056 | 0.193 | 0.055 | ... | ... | ... | ... |
| 3 | 0.021 | 0.047 | 0.056 | 0.098 | 0.394 | 0.416 | 0.462 |
| 4 | 0.005 | 0.056 | 0.060 | 0.049 | 0.309 | 0.248 | 0.184 |
| 5 | 0.059 | 0.110 | 0.103 | 0.122 | 0.078 | 0.146 | ... |
| 6 | 0.016 | 0.074 | 0.000 | 0.181 | 0.158 | 0.222 | ... |
| Av. | 0.033 | 0.082 | 0.073 | 0.154 | 0.237 | 0.328 | 0.323 |

weaning may have been of equal influence, although the average live weight of the two groups of pigs was about the same by 7 weeks of age. This restriction of growth may have hindered the volume production of saliva, which appeared to affect the concentration of amylase per unit volume. Whether the volume and total enzyme production of saliva have an age or weight relationship could not be determined in this investigation.

Raynaud and Rebeyrotte (9) reported that male mice gave higher salivary amylase values than female mice and that the effect of castration equalized these values; therefore, similar results might be expected with the baby pig. The data for the 49-day period indicate that the female and castrated male pigs developed similar salivary amylase levels, as there was a negligible difference in the average value between the sex classes.

As a check on previous sampling technique and to make observations on salivary amylase development of older pigs, three pigs from each of the three weight groups were selected at random. Two saliva samples were taken from each pig; one from the first noticeable flow of saliva and the second, 2 minutes after completion of the first collection. The results of this investigation, given in Table IV, show that the first sample of saliva had about one half of the activity of the second sample. The value for the 75-pound group of pigs was similar to that exhibited by the baby pigs at 7 weeks of age. A further decline in the salivary amylase activity appeared to occur with increasing live weight in the baby pig. This trend may continue in the heaviest of growing-finishing pigs.

**Table IV. Measurement of Salivary Amylase Activity[a] in Three Groups of Randomly Selected Pigs**

| Group | Pig No. | Weight, Lb. | | Saliva Samples 1 | 2 | Average |
|---|---|---|---|---|---|---|
| 50 | 6119 S | 51 | | 0.19 | 0.41 | |
| | 6117 S | 59 | | 0.15 | 0.16 | |
| | 6012 B | 54 | | 0.43 | 0.85 | |
| | | | Av. | 0.26 | 0.47 | 0.36 |
| 75 | 5715 S | 73 | | 0.44 | 0.82 | |
| | 5711 B | 78 | | 0.07 | 0.15 | |
| | 5795 S | 76 | | 0.02 | 0.05 | |
| | | | Av. | 0.18 | 0.34 | 0.26 |
| 130 | 5515 S | 130 | | 0.05 | 0.09 | |
| | 5648 S | 135 | | 0.15 | 0.20 | |
| | 5636 B | 115 | | 0.04 | 0.06 | |
| | | | Av. | 0.08 | 0.12 | 0.10 |
| | | Over-all av. | | 0.17 | 0.31 | 0.24 |

[a] Milligrams maltose equivalent formed per ml. of undiluted saliva.


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