isolated cat heart, and that this blockade of release is sufficient to account for the marked reduction in the positive inotropic effect produced by tyramine after phenoxybenzamine. Therefore, phenoxybenzamine does not block cardiac effects of this type of sympathomimetic amine by *beta*-receptor blockade but by blocking the release of catecholamines.

Before one may utilize these results to explain the blockade of the pressor effect of mephentermine, one must show that phenoxybenzamine blocks the cardiac release of catecholamines in the intact animal and that this release is the principal cause of the pressor effect.

Summary. The release of catecholamines produced by tyramine in the isolated cat heart is inhibited by phenoxybenzamine and GD-131 but not by phentolamine or dihydroergotamine. Inhibition of the release of catecholamines is associated with a marked reduction in the positive inotropic effect of tyramine. The possibility is suggested that β-haloalkylamines may interfere with the release of catecholamines *in vivo* as well as inhibiting the effects of catecholamines on effector cells.

The capable technical assistance of Miss Joan F. McGlinn is gratefully acknowledged.

1. Ahlquist, R. P., *Am. J. Physiol.*, 1948, v153, 586.

2. Eckfeld, D. K., Abell, L. L., Seifter, J., *J. Am. Pharm. Assn.*, (Scient. Ed.), 1954, v43, 705.

3. Goldberg, L. I., Cotten, M. de V., Darby, T. D., Howell, E. V., *J. Pharmacol.*, 1953, v108, 177.

4. Welch, G. H., Braunwald, E., Case, R. B., Sarnoff, S. J., *Am. J. Med.*, 1958, v24, 871.

5. Ellis, S., Perlmutter, J., Swaine, C. R., *Am. J. Med. Sci.*, 1960, v240, 396.

6. Fawaz, G., *Brit. J. Pharmacol.*, 1961, v16, 309.

7. Chidsey, C. A., Harrison, D. C., Braunwald, E., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 488.

8. von Euler, U. S., Floding, I., *Acta physiol. Scand.*, 1955, v33, Suppl. 118, 57.

9. Nickerson, M., Gump, W. S., *J. Pharmacol.*, 1949, v97, 25.

Received November 13, 1962. P.S.E.B.M., 1963, v112.

Histo-Physiological Studies on Chick Pancreas as Influenced by Feeding Raw Soybean Meal.* (28054)

H. C. SAXENA, L. S. JENSEN, J. MCGINNIS AND J. K. LAUBER
Department of Poultry Science, Washington State University, Pullman

Chernick *et al.*(1) reported that feeding raw soybean meal to chicks caused an enlargement of the pancreas and an increase in its proteolytic content. Distinct morphological changes in the hypertrophic pancreases of rats fed raw soybean meal were reported by Booth *et al.*(2). These authors suggested that the growth depression caused by ingestion of raw soybeans may be due to the excessive loss of critical amino acids contained in the pancreatic enzymes excreted in the feces. However, amino acid supplementation or increasing dietary protein levels failed to correct growth depression and pancreatic hypertrophy in chicks(3,4) or in rats(5). This investigation was conducted to explore the cause of pancreatic hypertrophy, using pilocarpine as an agent to enhance the flow of pancreatic enzymes.

Experimental. Male New Hampshire × Delaware chicks were maintained in a temperature controlled and ventilated laboratory. Chicks used in this study were reared in electrically heated, thermostatically controlled battery brooders with raised wire screen floors. Raw or heat treated soybean meal served as the source of protein in a semi-purified diet(6). Heat treated soybean meal was prepared by passing live steam over the meal, spread in shallow pans, in an autoclave for

* Scientific Paper No. 2276, Washington Agri. Exp. Stations, Pullman. Project No. 1678. Supported, in part, by funds from Medical and Biological Research by State of Washington Initiative No. 171.

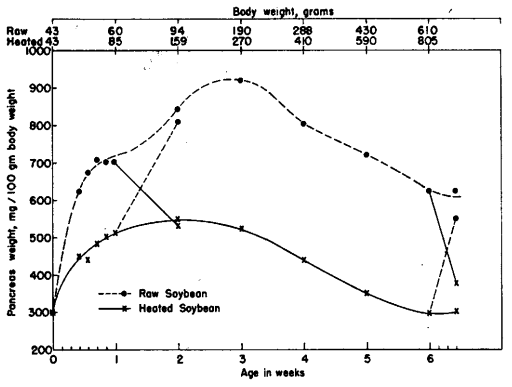

FIG. 1. Pancreas wt and body wt of chicks fed soybean meal. Body wt is depressed and pancreas is hypertrophic in birds maintained on raw meal. Pancreatic hypertrophy was reversible within 3 days after transfer of birds to the alternate diet.

30 min. Ten chicks were randomly assigned to each experimental group and 4 such groups were fed each of the experimental diets. Feed and water were supplied *ad libitum*.

Three chicks per treatment were randomly selected at various intervals, weighed individually, and then sacrificed for the purpose of pancreas measurements which were expressed as mg per 100 g body weight.

At one week of age 6 chicks receiving raw soybean meal diet were placed on heated soybean meal diet. A similar number of chicks receiving heated soybean meal were placed on raw soybean meal for a period of 1 week. At the end of this period, the above chicks were sacrificed and pancreas weights recorded. The same procedure was followed at 6 weeks of age with the same number of chicks, except that pancreatic measurements were taken 72 hours after the diet change was made.

At 2 and 6 weeks of age, chicks were starved for 10 hours and each treatment was divided into 2 groups. One group from each diet was injected intraperitoneally with 0.016 mg pilocarpine in 0.9% NaCl solution. Chicks were sacrificed 30 minutes after injection. A portion of the pancreas was then fixed in Bouin's solution for 24 hours, embedded in paraffin, sectioned at 8 μ and stained with hematoxylin and eosin. Another part of the pancreas was homogenized in cold 0.02M Sorensen's phosphate buffer at pH 6.9 and the homogenate used for estimation of amylase activity using the procedure of Noelting and Bernfeld(7).

Results. Pancreas weights (mg/100 g body wt) up to 6 weeks of age, along with the average body weight of chicks on 2 treatments, are given in Fig. 1. Results revealed pancreatic hypertrophy within 72 hours after chicks were started on raw meal. At one week of age, chicks returned to heated soybean meal diet showed, within a week, "normal" (control) pancreas weights. Again, at 6 weeks of age, the enlarged pancreas returned to "normal" within 72 hrs after changing chicks to heated soybean meal diet. Conversely, the pancreas was enlarged within this period for chicks changed from heated to raw soybean meal.

Amylase activity, in terms of maltose liberated in 3 min at 37°C per mg pancreatic nitrogen, is shown in Table I. At 2, as well as at 6 weeks, the chicks on both treatments showed high levels of pancreatic amylase activity after 10 hrs without food. Levels were slightly higher in chicks on raw soybean meal diet. Pilocarpine injection reduced amylase activity significantly in the pancreas of chicks on heated meal, but had no appreciable effect on pancreatic amylase activity in raw meal-fed chicks.

Histological examination of the pancreas after 10 hrs starvation of chicks revealed, as expected, an accumulation of eosin-stainable secretory material in the apex of acinar cells. The basal cytoplasm of the cells was intensely basophilic. Pancreatic enlargement in chicks fed raw meal appeared to be due mostly to an increase in the amount of accumulated zymogen in each cell. Mitotic figures were seldom seen; thus the pancreatic

TABLE I. Interference by Raw Soybean Meal Diet on Release of Pancreatic Amylase.

Type of soybean meal in diet	Chick age, weeks	Amylase activity, mg maltose/mg pancreatic N	
		No injection	Pilocarpine injection
Raw	2	45.7	44.8
	6	48.8	46.4
Heated	2	40.1	29.8*
	6	40.0	28.1*

* $P<0.01$.

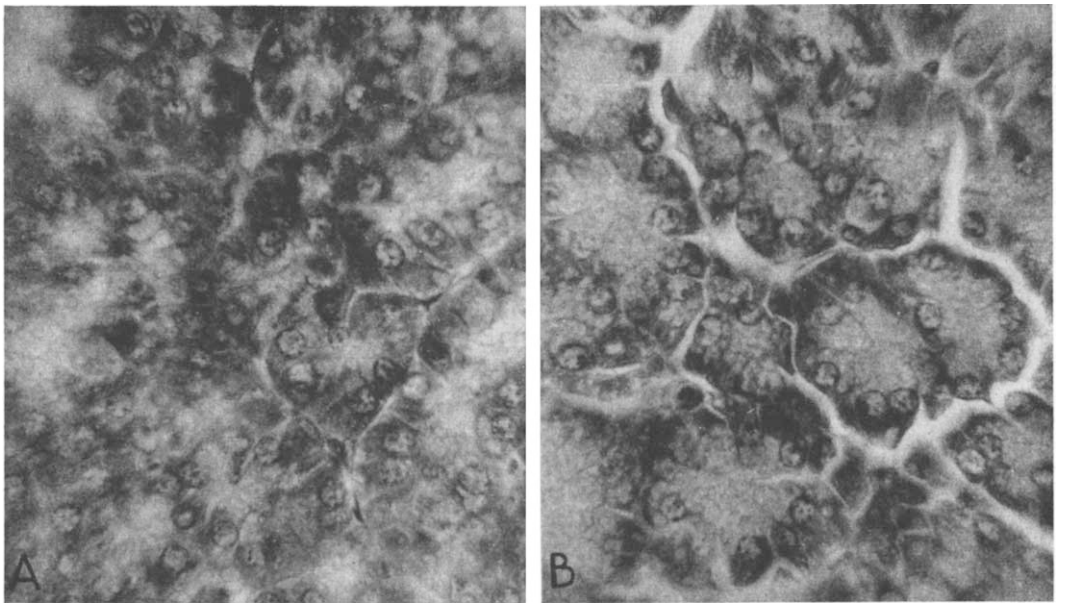

FIG. 2. Pancreas of 2-wk-old chicks after intraperitoneal injection of pilocarpine. The expected effect of this parasympathomimetic drug, to enhance flow of pancreatic enzymes, has resulted in zymogen depletion in the control pancreas (heated soybean meal diet, Fig. 2A). On raw soybean meal diet (Fig. 2B) the pancreas remains engorged with zymogen, in spite of pilocarpine stimulation. × 500.

enlargement appears to represent hypertrophy rather than hyperplasia. After pilocarpine treatment, secretory material was depleted in the control pancreas (Fig. 2A), while the pancreas of raw soybean-fed chicks remained engorged with eosin-stainable zymogen (Fig. 2B).

Discussion. It would appear from these results that the pancreatic hypertrophy in chicks fed raw soybean meal was caused by an accumulation of zymogen material in the acinar cells. Failure of the pancreas to respond to pilocarpine injection suggests that the raw soybean meal diet interferes in some way with the basic mechanism by which zymogen is released from the pancreatic cells.

Lyman and Lepkovsky(8) and Lyman(9) showed high enzymatic activity in the intestines of rats fed raw soybean meal. These authors have suggested that in the raw soybean-fed rat, excessive amounts of pancreatic enzymes were released and lost in the feces and that this mechanism could explain the poor weight gains of such animals. If the concept of fecal endogenous amino acid loss, rather than incomplete intestinal proteolysis, is to be accepted, then growth depression and pancreatic hypertrophy should be overcome by addition of amino acids. However, such was not the case in chicks(4) or in rats(5).

The results of the study reported here show, rather, that the hypertrophic pancreas of raw soybean meal-fed chicks is incapable of releasing its enzymes on demand. These observations derive further support from the findings that feeding raw soybean meal to chicks caused a complete inhibition of intestinal proteolysis(10) and depressed fat absorption(11). It is therefore suggested that the poor weight gain of raw soybean-fed animals can best be explained by a failure of the hypertrophic pancreas to supply the necessary digestive enzymes.

Summary. Pancreatic hypertrophy in chicks fed raw soybean meal is caused by an accumulation of zymogen material in the acinar cells. Failure of the pancreas to respond in the expected way to pilocarpine injection suggests that the raw soybean meal interferes in some way with the basic mehanism by which zymogen is released from the pancreatic cells. It therefore seems unlikely

that the growth depression in chicks fed raw soybean could be due to excessive fecal loss of critical amino acids contained in pancreatic enzymes.

1. Chernick, S. S., Lepkovsky, S., Chaikoff, I. L., *Am. J. Physiol.*, 1948, v155, 33.
2. Booth, A. N., Robbins, D. J., Ribelin, W. E., DeEds, F., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 681.
3. Saxena, H. C., Jensen, L. S., McGinnis, J., *J. Nutrition,* 1962, v77, 241.
4. ———, *ibid.,* 1962, v77, 259.
5. Barnes, R. H., Fiala, G., Kwong, E., *ibid.,* 1962, v77, 278.
6. Saxena, H. C., Jensen, L. S., McGinnis, J., *Poultry Sci.,* 1962, v41, 1304.
7. Noelting, G., Bernfeld, P., *Helv. Chim. Acta,* 1948, v31, 286.
8. Lyman, R. L., Lepkovsky, S., *J. Nutrition,* 1957, v62, 269.
9. Lyman, R. L., *ibid.,* 1957, v62, 285.
10. Alumot, E., Nitsan, Z., *ibid.,* 1961, v73, 71.
11. Nesheim, M. C., Garlich, J. D., Hopkins, D. T., *ibid.,* 1962, v78, 89.

Received November 13, 1962. P.S.E.B.M., 1963, v112.

Relationship of Estrogen and Vitamin K.* (28055)

P. B. RAMA RAO,† ANNA MARIA PAOLUCCI‡ AND B. CONNOR JOHNSON
Division of Animal Nutrition, University of Illinois, Urbana

During studies on the nutritional adequacy of irradiated beef, Metta *et al.*(1) observed the widespread occurrence of deaths due to hemorrhage in male rats. Vitamin K supplemented to the irradiated beef diets cured this hemorrhagic syndrome. It was observed that the female rat was more resistant to dietary Vit. K deficiency than was the male(2), and this difference was not due to lower food intake or growth rate of the female rat. Mellette and Leone(3,4) and others(5) have shown that the incidence of hemorrhagic syndrome associated with the feeding of irradiated beef was influenced by the age, sex and strain of the rat. Mellette(4) reported that administration of male hormones or castration of female rats results in an increased susceptibility to hypoprothrombinemia and hemorrhage while the converse is true for administration of estrogenic hormones and castration of the male animal.

We report here the effect of single doses of estradiol and of testosterone on prothrombin levels in rats(6) and chicks maintained on a synthetic Vit. K-free diet.

Another important aspect of this problem is the species difference in susceptibility to dietary Vit. K deficiency. The response of the chick as reported here is entirely different from that of the rat.

Materials and methods. Weanling rats of the Sprague-Dawley strain were maintained in tubular cages to prevent coprophagy throughout each experiment and were fed *ad libitum* a synthetic Vit. K-free diet(2,7).

Day-old chicks were fed a Vit. K-free soya protein-containing diet(2) *ad libitum* for 23 days. Plasma prothrombin times were determined essentially according to Quick(8). Commercial thromboplastin (Difco) was used in the rat experiments and acetone dehydrated chick brain powder was used as thromboplastin source in the chick experiments.

Results. 1. Estrogen administration, (a) Rat results. In a preliminary trial we observed that when estradiol was administered intramuscularly (at a dose of 1 mg/rat) to male rats after 24 days on the Vit. K-free diet, prothrombin times were restored to normal in 4 out of 6 rats, while the negative controls had the usual high prothrombin times

* This work was supported in part by a contract with the Surgeon General's Office, Dept. of the Army. The opinions expressed in this publication are those of the authors and not necessarily those of the Dept. of the Army.

† Present address: C111 Central St., Seshadripuram, Bangalore 3, India.
‡ Present address: Istituto Nazionale della Nutriz., Citta Universitaria, Roma, Italy.