ligan and Combs(12) who showed that vit. B_{12} activity is transmitted from the dam through the yolk of the egg. These workers also demonstrated that the diet of the dam had a direct influence on the amount of vit. B_{12} activity contained in the egg yolk.

The growth response of deutectomized chicks as compared with that of unoperated chicks in both experiments demonstrates that the yolk-removal operation had little or no effect on growth when the diet of the chick was adequately supplemented. Although no evidence was obtained in these trials to demonstrate the removal by deutectomy of nutrients other than vit. B_{12}, this procedure is considered to be of value as a means of reducing the initial "carry-over" of nutrients from the dam in day-old chicks.

Summary. An operative technic for the removal of the yolk from day-old chicks has been described. This procedure was developed to render chicks relatively free of the nutrients transmitted through the yolk from the dam. Observations on the growth rate of deutectomized chicks as compared with that of unoperated controls show that the operation had little or no effect on growth when an adequately supplemented diet was supplied. The results of these trials demonstrate that vit. B_{12} or a substance possessing vit. B_{12} activity was present in the yolk of the day-old chick and that it was removed by deutectomy.

This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service. The authors are grateful to Miss Josephine A. D. Blair, student in poultry husbandry at the University of Maryland, for her assistance in the deutectomy procedure. We are indebted to Merck and Co., Inc., Rahway, N. J., for providing crystalline vitamins, including vit. B_{12}; Lederle Laboratories, Pearl River, N. Y., for folic acid; Allied Mills, Inc., Portsmouth, Va., for soybean oil; Abbott Laboratories, North Chicago, Ill., for Haliver oil; U. S. Industrial Chemicals, Inc., New York City, N. Y., for DL-methionine; and Cerophyl Laboratories, Inc., Kansas City, Mo., for Protamone.

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Received November 27, 1950, P.S.E.B.M., 1951, v76.

Critical Evidence that Vagal Stimulation Does Not Release Gastrin. (18385)

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At least 2 mechanisms have been shown unequivocally to result in the gastric secretion of hydrochloric acid: (a) A vagally mediated mechanism, which is physiologically activated by the thought, sight, smell and ingestion of food; by stimulation of the vagus nucleus chemically with insulin-induced hypoglycemia; or by electrical stimulation of the vagal trunks directly; and (b) a chemical mechanism, which is physiologically activated by the presence of food in the antrum of the stomach or by distention of the antrum and which is followed by the release of the hormone, gastrin. These mechanisms have generally been considered to be independent of each other(1) although, in the normal course of ingestion and digestion of food, they may operate synchronously as well as successively. In recent years, however, the view has been advanced that a more direct relation exists between the nervous and the hormonal phases of gastric acid secretion. Uvnäs(2) has presented evidence from which he concluded that vagal stimulation during the neural phase of gastric secretion leads to the release of the antral hormone, gastrin. This hypothesis is extremely attractive, since it unifies the

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2. Uvnäs, B., *Acta Physiol. Scand.*, 1942, v4, Supplement 13.

various phases of gastric secretion by a common underlying neuro-humoral mechanism, and this possibility deserves careful consideration. Uvnäs employed anesthetized dogs and cats, and stimulated the cervical vagi electrically. His findings, as summarized by himself, fall into 2 main groups: First, the gastric secretion of HCl during vagal stimulation is greatly diminished if the pyloric region is deprived of its arterial blood supply, if the venous blood flow from that region is obstructed, if the function of the pyloric mucosa is depressed by cocainization, or if the pyloric portion of the stomach is resected. Second, cross circulation experiments were interpreted as indicating that a gastric secretory agent is liberated from the pyloric region during vagal stimulation. These findings have been restated by Kahlson, without essential addition of experimental evidence(3). Although Babkin and Schachter(4) failed to confirm the studies of Uvnäs, the suggestion that vagal stimuli release gastrin still continues to appear in the literature from time to time(5). More recently, Linde(6) has reported his repetition of Uvnäs' experiments using anesthetized cats, in which secretion of HCl induced by vagal electrical stimulation was diminished, but not completely abolished by (a) resection of the pyloric region, (b) ligation of the arteries supplying this part of the stomach, and (c) cocainization of the pyloric mucosa.

In the course of some experiments performed in this Laboratory(7), and designed to compare insulin and food as stimuli in the differentiation of vagal and non-vagal canine gastric pouches, evidence has been presented in part which bears directly on the question at hand. The pertinence of these studies to the problem of the vagal release of gastrin has been commented on by Grossman in his recent comprehensive review of gastrointestinal hormones(8) and by Linde(6). Because the significance of these experiments does not seem to have been generally recognized, and because of the questions raised by these latter reviewers, the present report was designed to furnish critical evidence that vagal stimulation does not release gastrin in the dog.

Methods. Four mongrel dogs with vagally denervated (Heidenhain) pouches, were employed in this study. One of the vagotomized pouches was prepared from a vagal pouch (Hollander-Jemerin type), one was converted from a Pavlov pouch, one was prepared by the conventional Heidenhain technic, the fourth was prepared as a Pavlov pouch but had suffered destruction of the vagal fibers supplying it, so that it actually was of the Heidenhain variety. Vagal stimulation was induced by the intravenous injection of 1.2-1.5 units of insulin in all 4 dogs, and by sham feeding in one dog following esophagostomy. All dogs were fasted for at least 20 hours before each experiment. The experiments were started only when the secretory activity of the pouch had reached basal anacidity, *i.e.*, 2 successive 15-minute samples were less than 0.2 ml in volume and contained no free HCl. Adequate hypoglycemia was considered to have been secured if the blood sugar was depressed to 50 mg/100 ml or below. Acidity was determined by colorimetric titrimetric procedures previously described from this Laboratory(9). Blood sugars were determined in duplicate by the method of Folin-Wu, as given by Reiner(10).

Results (Summarized in Table I). These 4 dogs were stimulated 11 times with an adequate insulin-induced hypoglycemia. In all instances, 0.1-0.3 ml of viscous mucus was obtained from the pouches during 2½ hours of collection, but at no time was even a trace of acid elicited.

Dog No. 118 was then subjected to esophagostomy, and vagal stimulation was induced

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4. Babkin, B. P., and Schachter, M., *McGill Med. J.*, 1944, v13, 127.

5. Glass, G. B. J., and Wolf, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 535.

6. Linde, S., *Acta Physiol. Scand.*, 1950, v21, Supplement 74.

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8. Grossman, M. I., *Physiol. Rev.*, 1950, v30, 33.

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TABLE I. Response of Vagally Denervated Canine Fundic Pouches to Vagal Stimulation of Main Stomach.

Dog No.	Exp. No.	Stimulus	Lowest blood sugar (mg/100 ml)	Pouch response			Comment
				Vol. (ml)	Character	Acidity	
46	74	Insulin	30	.2	Viscous mucus	0	Pouch responds to food and histamine by acid secretion
	78	"	29	.2	"	0	
74	45	"	42	.3	"	0	Same
	46	"	43	.3	"	0	
	47	"	30	.2	"	0	
108	63	"	31	.3	"	0	Same
	64	"	43	.2	"	0	
	68	"	36	.1	"	0	
118	75	"	20	.3	"	0	Same and simultaneously, main stomach responds to insulin hypoglycemia by acid secretion
	76	"	28	.1	"	0	
	79	"	18	.1	"	0	
118	80	Sham feeding		0			Same and simultaneously, main stomach responds to sham feeding by acid secretion
	81	" "		0			
	82	" "		0			
	83	" "		0			

by sham feeding. In 4 experiments with 30 minutes of sham feeding with cooked horse meat, there was again no secretory response in the vagally denervated pouch.

The ability of all 4 of these vagally denervated pouches to secrete acid on the same day under other conditions was demonstrated by copious acid secretion following histamine given subcutaneously. The ability of the pouch to secrete in response to chemical stimulation of the main stomach was proven by large amounts of acid in the pouch following a meat meal. The adequacy of the vagal stimulation of the main stomach under the test conditions was confirmed in one of the dogs (No. 118) by the presence of large amounts of free HCl aspirated from the main stomach during both insulin hypoglycemia and sham feeding.

Discussion. These experiments indicate clearly that intense vagal stimulation of the main stomach by insulin-induced hypoglycemia, or by sham feeding in the esophagostomized dog, did not result in the secretion of any acid whatever, by the vagally denervated (Heidenhain) pouch. Hence, it must be inferred that intense vagal stimulation of the main stomach with its pylorus and antrum intact did not release any hormonal or humoral factors capable of stimulating the secretion of acid by the vagally denervated pouch. Grossman(8), in accepting these results as constituting "crucial evidence in disproof of the possibility that vagal stimuli release gastrin," added the proviso, "unless the Heidenhain pouches studied by Jemerin were too refractory to respond to gastrin." Linde(6) recognized also that this type of evidence "suggests that stimulation of the vagal secretory nerves does not lead to release of gastrin. However, the possibility exists that gastrin is really released, but in such small amounts that the pouch does not respond." In this connection, it may be pointed out that the pouches were shown to be capable of responding both to histamine, and to food placed in the main stomach. Further, if the secretion of the main stomach following vagal stimuli as suggested by Uvnäs is due to released gastrin, there does not seem to be any valid reason why the pouch should also not respond to the postulated circulating gastrin. Although the implications of our experiments seem clear cut, the conflicting evidence(2,6) previously cited, has not been completely explained. Perhaps such explanation is unnecessary, since Babkin and Schachter(4), in repeating the Scandinavian workers' experiments, failed to confirm them, and demonstrated acid secretion by vagal stimulation in the complete absence of the pylorus and antrum. These workers emphasized the import-

ance of avoiding surgical trauma to the blood supply of the stomach.

This failure of vagal stimulation to release gastrin is consistent with the large body of experimental evidence which indicates that the mechanisms of the cephalic (neural) phase and of the gastric (hormonal) phase of acid secretion may operate quite independently of each other. This does not deny the possibility of additive or synergistic action between these two mechanisms. Nor does the present report controvert the evidence which indicated that local nervous pathways are involved in the release of gastrin by food in the stomach or by antral distention, but these are purely local(8).

Conclusion. The failure of vagally denervated canine gastric pouches to secrete acid in response to intense vagal stimulation of the main stomach constitutes critical evidence that vagal stimulation does not release gastrin.

Received November 28, 1950. P.S.E.B.M., 1951, v76.

Metabolism of Sea Urchin Spermatozoa and Induced Anaerobic Motility in Solutions of Amino Acids. (18386)

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The addition of any one of a number of different amino acids and peptides to suspensions of sea urchin spermatozoa in sea water has been found greatly to extend their functional life span(1). The period during which the spermatozoa retain their fertilizing capacity in dilute suspensions, can be increased more than 50-fold by means of these agents. During the period of prolonged life span the spermatozoa remain highly motile.

In contrast with mammalian spermatozoa, the metabolism of sea urchin spermatozoa is predominantly aerobic(2). In the absence of oxygen the spermatozoa soon become motionless(3,4). Ordinarily, the respiration of sea urchin spermatozoa decreases with time following dilution, from a high initial rate to practically zero, concomitant with loss of motility. It might, then, be expected that during the period of extended life and continuing motility induced by added amino acid or peptide the respiration would remain at a level similar to that of a freshly diluted suspension in ordinary sea water. The results of the present experiments show that such is not the case.

Material and methods. The sea urchins *Arbacia punctulata* (obtained at Woods Hole, Mass.) and *Lytechinus pictus* (obtained at Corona del Mar, Calif.) were used in this work. The semen was obtained by the KCl-injection method(5). Oxygen uptake was measured by means of Barcroft-Warburg manometers with conical, single-side arm vessels of 16 to 20 ml volume in which sperm suspensions of 3 ml final volume were generally used. Shaking was 85 to 95 c.p.m. and 3 to 4 cm stroke. The amino acid solutions were made isosmotic with sea water, at the pH of sea water, and diluted with sea water to the desired concentration. Since, in prolonged manometric experiments, growth of bacteria may be a factor in the results, determinations of their presence were made by microscopic examination and, in certain experiments, by platings on medium suitable for the growth of marine bacteria(6). In addition use was made of penicillin in many of the experiments, to check bacterial growth.

* Part of these investigations were carried out at the Marine Biological Laboratory, Woods Hole, Mass., with the assistance of Mr. Richard Glass.

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