Illinois Section.

University of Illinois, January 8, 1935.

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Effect of Eserine and Acetylcholine on Gastro-intestinal Motility in Normal Dogs.*

RICHARD FRANK, LEO ZIMMERMAN AND HEINRICH NECHELES.

From the Gastro-Intestinal Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

Eserine (physostigmine) has been extensively employed both in medicine and in physiology to stimulate intestinal motility. Choline and its esters have been utilized for the same purpose, but with less satisfactory results. Dale[1] and his coworkers originally described the effect of acetylcholine and several of its esters upon gastro-intestinal motility. Magnus and Le Heux[2] considered choline as the hormone which induces intestinal motility and subsequently, numerous attempts were made to treat paralytic ileus with choline and acetylcholine. Matthes[3] and Loewi and Engelhart[4] report the effect of acetylcholine, even in larger doses, as evanescent, for the compound is rapidly destroyed in blood by an esterase, which, however, may be successfully inhibited by eserine, even in dilution of 1:40,000,000. In the presence of eserine, acetylcholine remains active and potent in the circulating blood. Eserine is specific for the inhibition of this esterase, and with this datum as point of departure, we investigated the physiologic effects of a combination of acetylcholine and eserine upon post-operative ileus. In the course of that work, it soon became apparent that the literature furnished inadequate description of the effect of eserine upon gastro-intestinal

* Aided by the Louis L. Cohen Fund.

1 Dale, H. H., *J. Pharmacol.*, 1914, **6**, 147.

2 Le Heux, J. W., and coworkers, *Pflüg. Arch.*, 1919, **178**, 8, and following volumes.

3 Matthes, K., *J. Physiol.*, 1930, **70**, 338.

4 Loewi, O., and Engelhart, E., *Arch. f. exper. Path. u. Pharmak.*, 1930, **150**, 1.

motility in the normal dog, and nothing whatever concerning the intestinal motor response of the normal dog to eserine-acetylcholine. This paper presents such findings in normal dogs. The effects of acetylcholine-eserine upon the paralytic ileus of peritonitis will soon be published. To our knowledge, the combination of acetylcholine and eserine has not been used clinically to stimulate motility of the alimentary tract.

Dogs, averaging 25 lb. in weight, were tested under ether anesthesia. Blood pressure was registered in the usual way. Rubber balloons introduced in the stomach, small intestine, and colon, were connected to water manometers. Body temperature was maintained by a heated operating table.

Eserine salicylate and acetylcholine (Hoffman-La-Roche) were given intramuscularly. An average control period of three-quarters of an hour preceded each injection, which was administered when tonus and peristalsis were regular and minimal.

Four dogs each received 0.5 mg. of eserine, 12 dogs received 17 injections of 1 mg. of eserine, 4 dogs were each given 2 mg. of eserine, and one dog, 3 mg. All drugs were given intramuscularly. It was soon evident that quantities less than one mg. frequently did not produce any motility, and doses above 1.5 mg. often caused undesirable concomitant effects such as excessive salivation, increased respiration, and a tetanus-like intestinal contraction. One mg. of eserine did not yield these undesirable effects, and although not always followed by peristalsis of the gastro-intestinal tract, yet this dose in combination with acetylcholine was sufficient to elicit peristalsis quite regularly.

The intramuscular injection of 0.025 mg. acetylcholine had no effect on blood pressure or intestinal motility, but combined with one mg. of eserine, produced in several dogs a slight drop in blood pressure of 10-15 mm. of mercury, which disappeared after 2 to 3 minutes.

From Table I, it is evident that one mg. of eserine had evoked gastric motility in only 50% of the tests, whereas the combination

TABLE I.
Effect of Intramuscular Injection of Eserine (1 mg.) and Eserine (1 mg.) plus Acetylcholine (0.025 mg.) on Gastro-Intestinal Motility.

	Stomach		Ileum		Colon	
	Eserine	Eserine + Acet. Chol.	Eserine	Eserine + Acet. Chol.	Eserine	Eserine + Acet. Chol.
Increased Peristalsis	8	12	6	11	10	13
No effect	8	2	10	3	6	1

of this quantity of eserine with .025 mg. acetylcholine produced increased peristalsis of the stomach in 12 out of 14 trials. Eserine had no effect on the ileum in 10 out of 16 tests; but eserine-acetylcholine was without effect in only 3 of a total of 14 experiments. On the colon, eserine alone was ineffective in 6 out of 16 tests; the combination of eserine and acetylcholine, in only one among 14 trials.

To appreciate better the distinction between eserine alone and the combination eserine-acetylcholine, we might mention that, generally speaking, the intestinal motor response elicited by the latter is more pronounced, more decisive, and more generalized. In dogs in which eserine evoked no reaction, or at best, slight colonic movement, eserine-acetylcholine yielded widely disseminated motility of the entire gut.

The average latent period after eserine was 12 minutes for the stomach, 18 minutes for the ileum, and 11 minutes for the colon. For eserine-acetylcholine, the latent period was 14, 10, and 9 minutes respectively. The average duration in the effect of eserine on stomach, ileum, and colon was 21, 26, and 32 minutes respectively, as compared with an average duration after eserine-acetylcholine of 28, 35, and 34 minutes respectively.

From the above results, it is evident that 1 mg. of eserine, when given intramuscularly into normal dogs of 25 lb. average weight, does not regularly produce peristalsis. In several experiments, eserine proved completely ineffectual; in other instances, motility was manifest in only one or 2 segments of gut. If one mg. of eserine is combined with 0.025 mg. of acetylcholine, a rather constant effect is obtained on stomach, ileum and colon. Although it is known that acetylcholine *in vitro* induces very strong peristaltic activity of the gut and that a great deal of this effect is preserved *in vivo* whenever eserine is present to inactivate the blood esterase, we do not claim that the results of eserine-acetylcholine on intestinal motility are due only to the preservation of the acetylcholine by eserine. The effect must be considered as the resultant of the stimulatory action of both eserine and acetylcholine. Larger doses of eserine (2-3 mg.) rather consistently stimulate extensive intestinal motility. However, in this work, our primary concern has been to determine that dose of eserine which not only preserves acetylcholine in the blood, but avoids as well any undesirable concomitant effects.

Summary. Twenty dogs, under ether anesthesia were injected intramuscularly with eserine and eserine plus acetylcholine. One mg. of eserine or less did not regularly produce peristalsis of the stomach, ileum, and colon. When 0.025 mg. of acetylcholine was

combined with one mg. of eserine, peristalsis resulted, almost constantly in all 3 viscera. Intramuscular injection of larger amounts, 2-3 mg., of eserine regularly induced generalized intestinal motility but caused undesirable concomitant effects as well.

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Virus Adsorbed to Alumina-gel for Production of Antipoliomyelitis Serum in Sheep.

F. B. GORDON, JAMES A. HARRISON AND N. PAUL HUDSON.

From the Department of Hygiene and Bacteriology, University of Chicago.

Early in the history of experimental poliomyelitis it was demonstrated that the serum of both monkeys and humans, convalescent from the disease, had the property of neutralizing the action of the virus when the 2 were mixed *in vitro*. As therapeutic value has been ascribed to such serums a number of attempts have been made to produce antiserum with similar properties in large, poliomyelitis-refractory animals. In most instances horses have been employed and the results, although sometimes successful, have been irregular and seem to depend upon individual differences in responsiveness of the animals. The immunization of sheep has met with success in some hands (Howitt[1]) and failure or irregular results in others (Stewart and Haselbauer[2]). The production of poliomyelitis antiserum in goats has also been reported.

In these various types of animals, virus inoculations have usually been continued over a long period of time, in some instances years, before potent neutralizing serums were demonstrated. A method of immunization in which the time as well as the number of injections could be decreased would be a distinct improvement.

We were subjecting a sheep to a series of virus injections for the purpose of producing an antiserum when it occurred to us that virus adsorbed to a colloidal carrier might prove to be a better immunizing agent than the crude virus. There is evidence that the antigenicity of substances may be enhanced by injecting them in combination with a colloid. Hektoen and Welker[3] have reported the continued maintenance of a high precipitin level in rabbits following one injection of protein adsorbed to alumina-gel. Since the virus of

[1] Howitt, B. F., *J. Inf. Dis.*, 1932, **50**, 26.

[2] Stewart, F. D., and Haselbauer, P., *J. Exp. Med.*, 1928, **48**, 449.

[3] Hektoen, L., and Welker, W. H., *J. Inf. Dis.*, 1933, **53**, 309.