ness followed rapidly by prostration, cyanosis, convulsions and death. Practically all deaths occurred within 3 hours. Certain mice affected by the toxin recovered and looked healthy for several days; all subsequently died in 4-6 days with the usual signs of infection[9] and with numerous rickettsiae in their spleens and lungs.

The toxic factor occurred with some regularity in our preparations although the titers were never high. In 21 experiments 5 suspensions killed the majority of mice receiving a 1/20 dilution and 8 killed at 1/10, while 8 were non-lethal at the latter dilution. The toxicity of a suspension generally diminished after freezing and storage at —70°C or —20°C for a few hours but was consistently unaffected by storage at 0°C for 3-4 hours. These same general methods have failed in our hands to demonstrate toxicity in yolk sacs heavily infected with the Imphal, Calcutta,[9] Karp, Kostival and Host 21 strains of *R. orientalis*. Whether this indicates inherent differences in these strains or that growth is not profuse enough in eggs to produce detectable toxin remains to be determined.

Sera of normal men, rabbits and guinea pigs did not neutralize the toxin. In contrast, a 1/640 dilution of anti-Gilliam rabbit or guinea pig serum neutralized a lethal dose of toxin, and the patient's serum had a titer of 1/20, Table I. Antisera against only 4 of the 8 heterologous strains of *R. orientalis* contained even small amounts of antitoxin, yet each serum was known to protect mice against death by infection with at least 1,000 lethal doses of its homologous strain. Thus, results of toxin-antitoxin tests contribute to the other types of evidence[5,11] regarding immunological differences among strains of *R. orientalis*. Sera of guinea pigs convalescent from epidemic typhus, murine typhus, or spotted fever failed to neutralize the Gilliam toxin; their specific complement fixing titers[12] were 1/240, 1/320, and 1/320, respectively.

Conclusion. Yolk sacs of embryonated eggs infected with the Gilliam strain of *R. orientalis* contain a specific toxin lethal for mice. The toxic substance is readily neutralized by Gilliam antiserum, but antisera against 8 other strains of *R. orientalis* contain only small amounts of antitoxin or none.

[12] Plotz, H., Wertman, K., and Bennett, B. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 76.

15400

Effect of Distention of Jejunum upon Tonicity of the Cardia of the Dog.

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This study was undertaken to determine if there are any extrinsic reflex effects upon the tonus and motility of the cardia during distention of the jejunum.

Methods. Records were obtained from 4 dogs over a period of 3 months. A modification and extension of the method outlined by Zeller and Burget[1] for recording motility of the cardia in the unanesthetized dog was used. A jejunal Thiry fistula was prepared in each dog, then an esophagostomy was made following recovery from the first operation.

About a week after the esophagostomy was performed, the training of the dogs was begun. They were trained to lie quietly on a table with the balloons in position while records of motility were obtained. Three balloons were in place and simultaneous tracings made on a smoked drum. The upper balloon was a 2 cm segment of condom tied over a rather rigid 14 Fr. catheter and inserted about 4 or 5 cm into the esophageal fistula and tied in place. It was attached to a

[1] Zeller, W., and Burget, G. E., *Am. J. Dig. Dis.*, 1937, **4**, 113.

mercury manometer. The balloon for recording cardial activity consisted of a 3 cm segment of condom enclosing soft sponge rubber on the end of a 16 Fr. catheter. The soft sponge rubber served the purpose of keeping the balloon open and sensitive to decreases as well as increases in pressure, and helped to keep the cardial balloon in proper position. The sponge rubber was fashioned with sharp scissors, was 3 cm long, 1 cm in diameter, and tapered slightly at both ends. The catheter occupied completely the long axis of the balloon. After insertion it was tied in place at the esophageal fistula and was attached to a sensitive Becker tambour. This caused no discomfort to the dog and did not initiate secondary esophageal peristalsis after the first 2 or 3 insertions in the training period. A 4 cm condom balloon was placed over soft rubber tubing, tied in place and attached to a mercury manometer for recording motility in the loop of jejunum brought to the surface in the first operation.

In making a recording, the cardial balloon was first passed through the esophageal fistula and down the esophagus until it met the moderate resistance of the cardia. About 1 cm more of catheter was allowed to enter the fistula and then the catheter was marked at its point of emergence from the fistula and tied in place. About 3 cc of air were introduced through a Y connection into the tubing connecting the cardial balloon with the Becker tambour. Next the upper esophageal balloon was introduced and tied in position 4 or 5 cm below the fistula and water admitted to the recording system through a Y connection until the mercury manometer was at zero pressure. This same Y connection was used later for introducing 10 cc of water into the balloon to initiate a peristaltic wave. The third balloon was placed in the jejunal loop and 3 to 5 cc of water introduced depending upon the level of motility of the loop. A Y connection in this system allowed for increasing the intraluminal pressure in the jejunum to determine the effect of this on the cardia.

Results. When the dog swallowed spontaneously, or when 10 cc of water were injected fairly rapidly into the upper esophageal balloon and then withdrawn, the cardia relaxed in from 1 to 4 seconds while the peristaltic wave was still high in the esophagus. The maximum relaxation was reached in 5 to 10 seconds or at about the time that the peristaltic wave had reached the cardia. These results are similar to those of Zeller and Burget.[1] Following relaxation, there was a rise in the tonus of the cardia and then a return to normal.

Rhythmic contractions of the jejunum were of large amplitude and were without superimposed periodic increases in pressure in one of the 4 dogs. The others exhibited the more typical variations in amplitude, but at no time was there observed a relaxation of the cardia in response to these spontaneous increases in jejunal pressure. On 2 or 3 occasions, there was noted relaxation of the cardia similar to that produced by intestinal distention, as described below, but without any apparent reason.

It was found that the tonus of the cardia is comparatively insensitive to pressure changes in the jejunum. However, it was demonstrated that repeated, moderate or severe distention of the jejunum may elicit relaxation of the cardia. After recording several typical responses of the cardia to stimulation of the esophagus by distention of the upper balloon, the intestinal balloon was distended sharply with from 4 to 7 cc of water. The amount of water used depended upon the response of the dog, an attempt being made to avoid producing evidence of pain. The pressure was maintained for from one to 3 seconds and then withdrawn. Variations of this procedure were tried in which pressure was maintained for 10 seconds and again in which pressure was alternately raised and lowered in quick succession 4 to 5 times. Neither of these latter 2 variations seemed to alter the result obtained by the first method. The amount of water necessary to elicit relaxation of the cardia varied from dog to dog and from time to time in the same animal. The only obvious factor influencing this amount was the original pressure within the jejunal balloon. When this pressure was high, the volume necessary to elicit relaxation of the cardia was materially reduced. Thus the original tonus of the segment of intestine determines

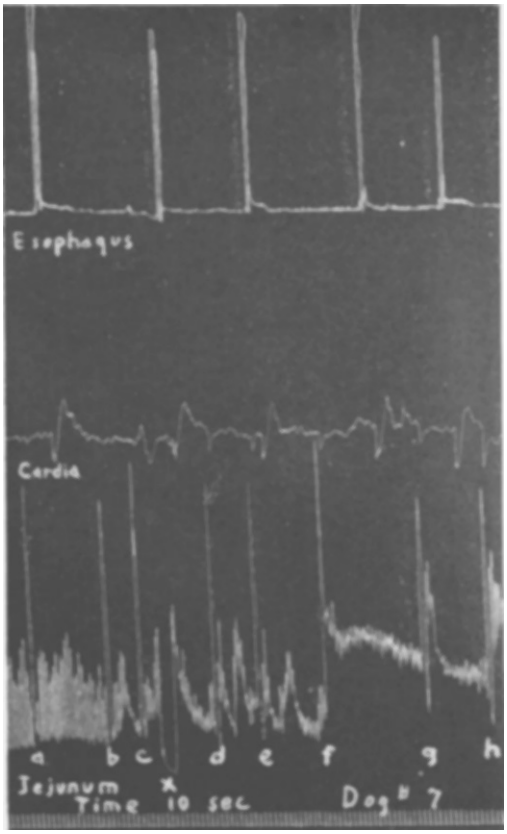

Fig. 1.
Each of the 5 upstrokes on the upper record shows a brief period of pressure produced by introducing 10 cc of water into the balloon in the esophagus. In each case a typical relaxation-contraction pattern was elicited in the cardia. The balloon in the jejunal segment was briefly distended with 7 cc of water at a, b, c, d, e. At f 7 cc were injected and 2 cc removed. At g and h 4 cc were injected and removed. At x the balloon slipped out of the jejunal fistula and was reinserted.

to some extent the sensitivity of this reflex. It usually was found that relaxation of the cardia would not occur with the first 2 to 7 distentions of the intestinal balloon in any experiment, but subsequent distentions with the same volume of water would produce first only slight or questionable decrease in tonus and then unmistakable relaxation of the cardia. Once the correct amount of distention had been determined and the loop had been "sensitized" by preliminary distentions, the relaxation of the cardia could be elicited nearly every time.

The maximum decrease in tonus of the cardia as a result of intestinal distention was usually at least half as great as that obtained as a part of the act of deglutition, and it often approached the amplitude of the latter. Typically, the relaxation occurred in from 2 to 5 seconds, was maximal in 3 to 7 seconds more, and the tonus was back to normal in 15 to 30 seconds. In most cases the relaxation was not followed by a marked rise above the normal tonus level such as that seen in the complexes resulting from spontaneous swallowing or from esophageal distention. The differences in the responses of the cardia to esophageal and to jejunal distention are illustrated in Fig. 1.

No evidence of discomfort or nausea was noted in the dogs at any time as a result of simple distention of the intestinal balloon with small amounts of water and at low pressures. When the pressure had been increased by retaining 4 or 5 cc of water in the balloon, subsequent distentions resulted in a stretching movement of the animal which distorted the record due to a sharp upward deflection of the lever from the cardial balloon. With further increases of pressure up to 80 or 90 mm of mercury the dogs showed evidences of nausea, such as salivation and licking movements, and would eventually vomit if this pressure were maintained. Prolonged relaxation of the cardia of about 60 seconds duration was produced preceding and accompanying signs of nausea in one animal in which the intestinal balloon was distended to the extent of 60 mm of mercury and again to 80 mm of mercury.

The possibility that intestinal distention may alter the motility of the cardia was studied by a second method. The pressure in the intestinal balloon was increased in steps of 10 to 30 mm of mercury by the introduction of 3 to 5 cc of water at a time. Between each increase in pressure the upper esophageal balloon was inflated 2 or more times, initiating a swallow reflex. In nearly all cases the cardia responded with a typical relaxation-contraction pattern upon inflation of the esophageal balloon even with pressures within the jejunal balloon as high as 80 to 90 mm of mercury.

Comment. Intestinal distention is capable

of provoking vomiting, and relaxation of the cardia has been assumed to precede or accompany the vomiting act. These experiments indicate that the cardia is not easily influenced by alterations in pressure in a short isolated jejunal segment. However, relaxation of the cardia may occur in the dog with a moderate, localized jejunal distention without accompanying signs of nausea or other distress. The brief time interval between jejunal distention and relaxation of the cardia necessitates the assumption that this is a reflex. The pathways for the reflex have not been studied. However, the surgical procedure for preparing the jejunal fistula eliminates the possibility of this being an intrinsic reflex. If intrinsic pathways were present it is possible that they might contribute to the effect on the cardia.

It is possible, as in the case of the intestino-intestinal inhibitory reflex,[2] that this reflex could be elicited with less pressure if a longer segment of jejunum were distended. It is also quite possible that the cardia may be influenced more readily by duodenal than by jejunal distention.

Summary and Conclusions. Experiments were undertaken in unanesthetized dogs to study the effect of distention of a segment of jejunum, in the form of a Thiry fistula, upon the tonus of the cardia and upon the relaxation of the cardia resulting from swallowing or from esophageal distention. It was found that the cardia is not easily influenced by changes in jejunal pressure. However, repeated moderate distention of a short jejunal segment may produce a decrease in tonus of the cardia without evidences of nausea or other distress. Secondly, it was found that distention of a jejunal segment does not alter the relaxation-contraction pattern of the cardia which is produced by the swallowing act or by esophageal distention.

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15401

Isolation of an Antibiotic Agent Derived from a *Phycomyces* Active *in vitro* Against *Trypanosoma equiperdum*.*†

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The more important antibiotic agents obtained from microorganisms have attracted attention largely because of their antibacterial properties. Any concomitant activity against fungi, actinomycetes, protozoa, or viruses has usually been observed only subsequently, and coincidental to the selective antibacterial effects. This is true, for example, of the antifungal action of streptothricin,[1] the inactivation of certain bacteriophages by streptomycin,[2] and the parasiticidal activity of tyrothricin.[3] Actinomycin and streptothricin prolonged the lives of mice infected with *Trypanosoma equiperdum*.[4] *Trichomonas vaginalis* has been killed *in vitro* by gram-

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