controls. Two showed *B. coli* in the mesenteric lymph gland and this bacillus was isolated from the liver of one dog. Cocci groups—mostly enterococci—were cultured from the majority of mesenteric lymph nodes and liver of all dogs.

Mechanical irritation due to the introduction of a vaselined catheter and 10 cc. of sterile saline causes the appearance of viable *B. coli* in the internal organs if cultured within 15 minutes. After 45 minutes *B. coli* cannot be cultured from these same organs. We could not demonstrate any difference in permeability between *B. prodigiosus, B. murii,* and *B. welchii.*

The accompanying graph represents the 15 minute time interval after mechanical manipulation only, after *B. prodigiosus* in saline and after *B. prodigiosus* in alkaline and acid phosphate buffered solutions. The differences between the latter 2 solutions are very striking. Rats have also been used and the observations made upon dogs have been substantiated. The same technic and the same test bacteria were used. Fifteen minutes after mechanical manipulation associated with the introduction of a thin, oiled catheter through the anus and injection of 10 cc. of saline, viable bacteria can be demonstrated within certain organs. Endogenous bacteria are present when sterile saline is used, exogenous bacteria are present if they were suspended in the saline.

5945

Influence of Saponin upon Bacterial Flora, H-ion Concentration and Permeability of the Intestinal Tract.

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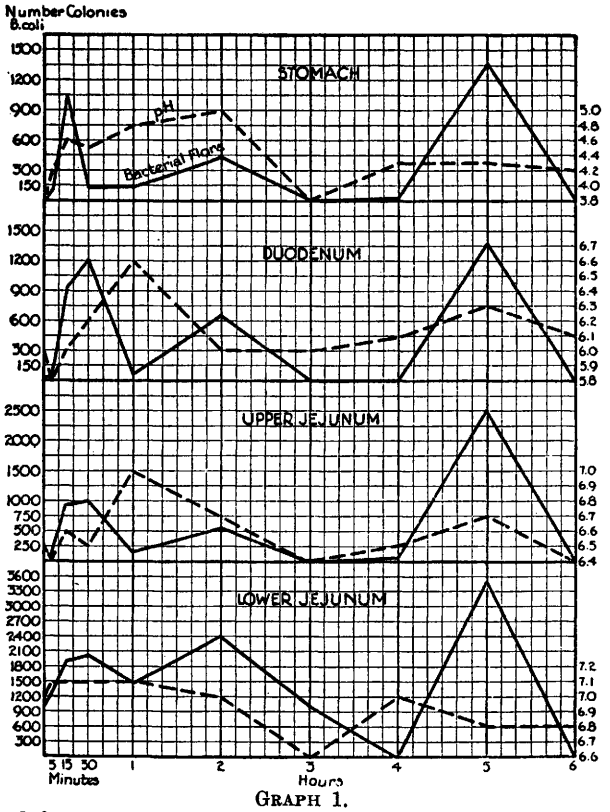
Kaufman[1] showed that the oral ingestion of small doses of saponin caused a sudden alteration in the bacterial flora and the acid-base equilibrium of the contents of the stomach and small intestine of mice. His work was part of a study upon the increased susceptibility of rodents to enteritides infections. We have extended Kaufman's experiments to dogs. In addition to the intra-intestinal changes we have studied the distribution of *B. coli* in the organs of these animals.

[1] Kaufman, I., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 21.

Thirty-four dogs were used. Two cm. of a 20% aqueous solution of saponin was administered by stomach tube. The animals were killed at various time intervals. The hydrogen-ion concentration of the contents of the stomach, duodenum, upper and lower jejunum was determined by Heilig's capillary comparator, and the distribution of *B. coli* was obtained by culture upon Endo media. The lymph glands of the splanchnic region, liver, kidney, spleen and lungs were also examined for the presence of *B. coli* by taking pieces from these organs, putting them into flasks of broth for 18 hours, then spreading 5 drops from these broth cultures on Endo media plates. The plates were read in 48 hours. No bacteria were administered in these experiments. The *B. coli* cultured in organs were endogenous strains.



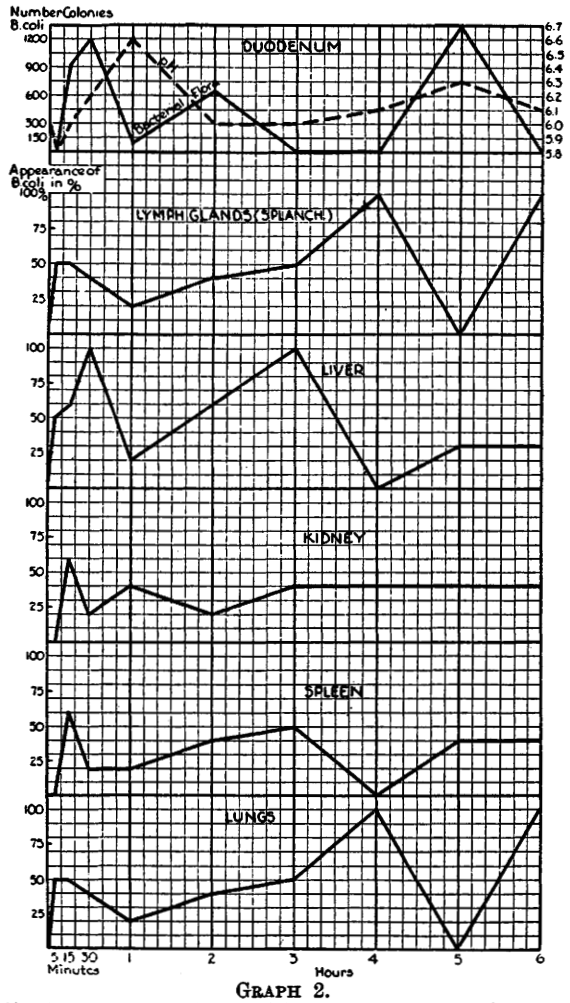
GRAPH 1.

Ordinate: left, number per swab of *B. coli* grown from contents of intestinal segments as indicated. Right, pH of same contents.

Abscissa: time as indicated.

The broken line represents H-ion concentration curve. The solid line represents *B. coli* count.

Two cubic centimeters of 20% saponin put in stomach at beginning of experiment.



GRAPH 2.

Ordinate: % of animals showing positive *B. coli* culture of organs indicated.
Abscissa: time as indicated.
The upper (duodenum) curve is reproduced from Graph 1 to indicate relationship of the intra-intestinal contents and organ cultures.

Graph 1 shows that saponin causes a change within the acid-base balance and the bacterial flora in dogs similar to those reported by Kaufman for rats. Graph 2 shows that there is associated with the intra-intestinal changes the sudden appearance within certain organs, mesenteric lymph node, liver, kidney, spleen, and lungs of viable *B. coli.*

The oral ingestion of saponin causes a sudden change in the acid-base equilibrium and in the endogenous bacterial flora. This is associated with the appearance of viable *B. coli* in the organs of

these animals. The wave-like cycle of these chemical and bacterial changes reported here are characteristic of autonomic nervous system disturbances.

5946

Influence of Gastric Acidity upon Viability of Bacteria in Isolated Stomach.

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Johnson and Arnold[1] in a series of experiments on dogs with a non-leaking gastric fistula have found that exogenous bacteria (*B. prodigiosus*) lose their viability in the presence of free hydrochloric acid and when the gastric contents were alkalinized with Na_2HPO_4 buffer solution these previously non-viable exogenous bacteria regained their viability. This phenomenon was so constant that we decided to set up acute experiments in which we could entirely separate the stomach from the duodenum. By this technic we could completely exclude the possibility of any regurgitation from the intestine.

The experiments on dogs were conducted in the following manner. The abdomen of an animal, under nembutal anesthesia (1 cc. of 3% solution per kilo intraperitoneally), was opened and the pyloric part of the stomach with the duodenum brought up to the surface. A series of ligatures was put around the pyloric end of the duodenum through the wall of the duodenum to avoid a hemorrhage into the stomach and then the duodenum was severed about 1 cm. below the ligatures. In such a manner we produced a wide and non-bleeding opening into the stomach which facilitated the securing of specimens from the gastric contents. All the specimens were taken by sterile Wright pipettes.

First, we determined the pH (LaMotte hydrogen-ion testing set) of the gastric contents, then introduced into the stomach a suspension of *B. prodigiosus* (washings of a 24-hour growth on agar plate in 20 cc. sodium chloride solution) and simultaneously injected 1.5 mg. of a histamine intravenously. At certain time intervals (see

[1] Johnson and Arnold, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**.